EPIDEMIOLOGY OF HAEMOPARASITE INFECTION AND THEIR EFFECTS ON HEMATOLOGICAL PARAMETERS IN SCAVENGING CHICKENS OF WEST GOJJAM ADMINISTRATIVE ZONE, AMHARA REGION, ETHIOPIA

MSc Thesis

By

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A Thesis submitted to the College of Veterinary Medicine and Animal Sciences, University of Gondar, for partial fulfillment of the requirements for the Degree of Master of Science in Veterinary Parasitology

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Everything is rubbish unless his blessings

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LIST OF ABBREVATIONS

BRDVL	Bahirdar Regional Diagnostic Veterinary Laboratory
CSA	Central statistics Authority/Agency
DNA	DeoxyriboNeuclic Acid
EDTA	Ethylene Diamine Tetracetate Acid
Hb	Hemoglobin
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hem Concentration
NCD	Newcastle Disease
PA's	Peasant Associations
PPM	Parts per million
Rpm	Revolution per minutes
TRBC	Total Red Blood Cell count
TWBC	Total White Blood Cell count
WGARDO	West Gojjam Agricultural and Rural Development Office

ABSTRACT

A cross-sectional study was undertaken with the aim of estimating the prevalence, identifying parasite species and assessing associated risk factors for the occurrence of haemoparasites in indigenous and cross breeds backyard chickens in selected districts of West Gojjam zone, Amhara region, Ethiopia from October 2016- May 2017. A total of 384 blood samples were collected and examined for the presence of haemoparasites using thin blood films. Out of these samples, 71 (18.5 %) of them were infected with haemoparasites. Four species of haemoparasites were identified, namely *Aegyptienella* spp., *Haemoproteus* spp., *Leucocytozoon* spp. and *Plasmodium* spp.. Highest prevalence of haemoparasite infection was recorded in midland (22.3%) compared to highland (10.9 %) areas with a statistical significant difference ($p<0.05$) between them. Statistical significance variation ($p<0.05\%$) was observed in haemoparasite infection among districts, with highest prevalence in S/Achefer (27.3%) followed by B/Zuria (17.2 %) and the lowest in Mecha (10.9%) districts. Age and management system of chickens were significantly associated ($p<0.05$) with haemoparasite infection. While sex and breed did not show significant difference between their counter parts. Based on haematological analysis, red blood cell count and level of hemoglobin was showed a statistical significant ($p<0.05$) difference between infected and non-infected chickens. The results obtained in this study suggested that haemoparasite infection in chickens is prevalent in the study area. Hence, appropriate control measures should be implemented to solve the existing problem.

Keyword: *Chicken; Cross-sectional; Haemoparasite; Hematology; Questionnaire Scavenging*;

1. INTRODUCTION

Ethiopia has a huge population of poultry estimated about 57.0 million with domestic chicken, cross and exotic breeds mostly kept in urban areas representing 95.86 %,2.7% and 1.35 %, respectively (CSA, 2015a). As the total population of poultry in Ethiopia, 99% are raised under extensive management system, whereas 1% is under semi-intensive and intensive management system (Tadelle *et al*., 2000).

Poultry production in most tropical countries is based on the scavenging rearing system. This system exposes poultry to a variety of parasites (Sehgal *et al*., 2006). However, the most prominent problem in relation to village poultry production is their high mortality within the first year after hatching (Sabuni *et al*., 2011). The chicken were kept around the houses in all over the world to produce eggs, source of food and for selling to get cash. These productive faunas are exposed to numerous parasitic diseases such as haemosporidians which are known to be highly pathogenic to domestic poultry with mortalities as high as 90% (Hassan, 2015).

Avian haemoparasites are microscopic, intracellular, some extracellular, single celled eukaryotic parasites found within the blood cells and tissues of the host (Donovan *et al.,* 2008*).* Hemoparasites are also common blood parasites of reptiles, birds and mammals with some stages of development in both tissues and circulating blood cells of the infected hosts (Archawaranon, 2005). Haemoparasitism in birds is usually caused by four genera of the Apicomplexan parasites of the family Plasmodiidae of the genus; *Leucocytozoon, Haemoproteus, Plasmodium* and *Trypanosoma* (Donovan *et al*., 2008). Most species of the hemoparasites are host specific and they are restricted to birds of the same family (Ozmen and Haligur, 2004). The commonly identified and most important species of poultry hemoparasites are; *L. caulleryi*, *Haemoproteus* spp., *P. gallinaceum* and *T. avium* and *A. pullorum* (Sehgal *et al*., 2006; Zidkova *et al*., 2011). The blood sucking insect vectors transmitting the infective stage of the parasites from infected to the non-infected while blood meal (Valkiunas, 2005). Poultry blood parasites are distributed globally in the temperate and tropical climates, but not found in Antarctica. This is due to the presence of diverse habitats of their vectors (Svensson-Coelho *et al*., 2014).

Hemoparasites can produce great hematological and histopathological changes in the host poultry organs and tissues. The most common gross pathologic changes are enlargement of the liver, spleen and lungs. The liver becomes dark brown to black, when there is extensive haemolysis (Lapointe *et al*., 2012). Microscopic changes of the lesions are indicated by impression smears of the affected organs; schizonts can be present in the liver, spleen, kidney, heart, skeletal muscle, brain, spinal cord and eyes (Atkinson *et al*., 2008). Gross and microscopic lesions can cause mild to severe necrosis of the liver, spleen and kidney; degeneration of myocardium and skeletal muscle; pulmonary edema, interstitial pneumonia, haemorrhages, lameness, reproductive failure, declined in meat and egg production and the disease is fatal, specifically in young poultry (Peirce, 1989).The total cost of Global nature destruction is estimated to be $ 4,500 billion annually. Most of this biodiversity loss is provoked by intentional and unintentional introductions of nonindigenous species becoming invaders in new ecosystem (The Economics of Ecosystem and Biodiversity) (Marzal., *et al* 2011).

Poultry is the most important animal species in West Gojjam Zone, Amhara region both for nutritional value and cash income generation than other animals meanwhile they are the main resources especially for deprived households. Furthermore, chicken raising system is now becoming more strengthened than the previous in which haemoparasitic disease in the area of emphasis. Despite several researchers undertake research on chicken haemoparasites in different parts of Asian countries, African countries and Ethiopia (Poulsen *et al*., 2000, Njunga, 2003, Permin *et al*., 2002; Sehgal *et al*., 2006; Sabunni *et al*., 2011 and Emebet, 2017). The status of Haemoparasitic diseases of chickens not well known and documented in West Gojjam zone of Amhara region and this necessitates research and investigation.

The occurrence and prevalence of poultry haemoparasites among domestic chicken, resident wild birds and migratory avifauna requires constant monitoring in order to investigate and minimize potential outbreaks that may be detrimental to the local poultry industry. Therefore; identification and defining of the haemoparasites prevalence and identification of the parasite at species level were done by obtaining of the blood samples from the live poultry by processing the appropriate parasitological techniques of prepared blood smears and defining the hematologic findings alongside on questionnaire survey to assess the management system of poultry in the study area.

Therefore, the objective of the study were:

General objectives:

- ❖ To determine the prevalence of poultry haemoparasites based on blood sample examination and to assess the influence of host related factors in different agroecological areas of the West Gojjam zone.

Specific objectives:

- To determine the prevalence of chicken haemoparasite in the study area.
- To define the risk factors associated with parasite occurrence.
- To evaluate and compare the haematological findings of the infected chicken
- To investigate the management system of the chicken in the study area.

2. LITERATURE REVIEW

2.1 Etiologic Agents

The causative agents are grouped under phylum: Apicomplexa, order: Achomatorida, family: Leucocytozozoidae, genus: *Leucocytozoon*, species, *L. caulleryi, L. smith* and *L. simondi* (Ozmen and Haligur, 2004). Under the phylum: Apicomplexa, Order: Haemosporida, family: Haemoproteidae, genus: *Haemoproteus*, species, *Haemoproteus* (*parah.*) *micronuclearis*, *Haemoproteus* (p*arah.*) *cleofascialis and Haemoproteus* (*parah.*) *paranucleophilus* (Iezhova *et al*., 2011). Under the family: *Plasmodiidae*, genus *Plasmodium* species which affect chickens are *P.gallinaceum*, *P. juxtanucleare*, and *P. durae* (Friend and Franson, 2001; Sehgal *et al*., 2006). Avian Trypanosomosis is caused by the genus *Trypanosoma*, with common species affecting chickens are *T.benneti, T.corvi, T.avium, T. gallinacium, T. culicavium, T. polygranularis and T. anguiformis* (Sehgal *et al*., 2006 and Zidkova *et al*., 2011). Aegyptianellosis is a rickettsial disease which is caused by genus *Aegyptianella pullorum.* The causative agent is transmitted with soft ticks of fowl. *A. pollorum* is the only species which can cause high morbidity and mortality in domestic and wild chickens (Tarello, 2005).

2.2 Morphology of Haemoparasites

2.2.1 Morphology of *Plasmodium* Species

Infections of *Plasmodium* are characterized by the presence of several stages of the organism within the erythrocytes of the host. Remarkably, gametocytes and schizonts (containing merozoites) may be recognized. Both gametocytes and scizonts of *Plasmodium* can be round, signet ring, oval or irregular in shape (Soulsby, 1982).

2.2.2 Morphology of *Haemoproteus* Species

Gametogony of *Haemoproteus* occurs within erythrocytes, whereas schizogony occurs within endothelial cells. Consequently, only gametocytes are observed within erythrocytes in contrast to *Plasmodium* species. Multiple gametocytes within a single erythrocyte are commonly observed. In general gametocytes of *Haemoproteus* spp. have a distinct peripheral outline of cytoplasm that

contains variable amounts of a yellow to black-brown granular pigment that stains pale blue to rose color (Pierce, 1989).

2.2.3 Morphology of *Leucocytozoon* Species

Abundant species of *Leucocytozoon* have been recognized from many families of avian hosts. The gametogony of the *Leucocytozoon* occurs within hematological cells, whereas the schizogony occurs in various parenchymatous and endothelial cells. The gametocytes of *Leucocytozooons* are highly pleomorphic with exceptions of some species exhibiting both fusiform and only round forms. The mature gametocytes have the size measuring 10-15 µm round or oval in shape and found in immature and mature erythrocytes. The size of the host cell is approximately 20 µm in diameter. Full grown gametocytes press the nucleus of infected host cells out (Pierce, 1989).

2.2.4 Morphology of *Trypanosoma* Species

The organism is detected with blood films and aided by centrifugation of a sample of blood in a capillary tube and examination of the buffy-coat preparation. The most recognizable stage of the parasite is trypomastigotes stage. Structurally, trypomastigotes have a blade like elongated shape that tapers to a posterior flagellum and a pointed anteriorly (Murray *et al*., 1983).

2.2.5 Morphology of *Aegyptienella* Species

Normal chicken red blood cell does not show inclusion bodies in the cytoplasm of the red blood cell. Microscopic detection of the organism is look like round, oval and clover- like inclusions. The initial bodies occurs in form of trophozoites. Which measures 0.5-1.0 µm size. This organism can be seen either inside or outside the cytoplasm of the red blood cells membrane with erythrocytic vesiculation is the mode of entrance or exit of initial bodies from the red blood cells (Tarello, 2005).

2.3 Distribution of Haemoparasites

Avian haemoparasites are distributed globally in the temperate and tropical climates, but not found in Antarctica. This is due to the presence of diverse habitats of their vectors. From the various studies of *Haemosporidian* diversity, it is know that some parasites have worldwide distributions, and others

appear to be localized to specific regions and habitats. In general the genus *Plasmodium* appears to be more cosmopolitan than *Haemoproteus*, but with lower ancestry diversity (Svensson-Coelho *et al*., 2014).

Table 1: Prevalence and Distribution of Chicken Haemoparasite

Country	**Prevalence in %**	**Reference**
Ghana	35.0	Poulsen *et al*. (2000)
Zimbabwe	32.0	Permin *et al*. (2002)
Kenya	79.2	Sabuni *et al*. (2011)
Iraq	78.2	Shadan (2013)
Iraq	76.0	Hasson (2015)
Nigeria	12.0	Opara (2016)
Ethiopia	43.4	Emebet (2017)

2.4 Transmission of Haemoparasites

Haemosporidians are transmitted from infected to uninfected chicken by a variety of biting flies that serve as vectors, including mosquitoes, black flies, ceratopogonid flies, biting midges or sandflies and louse flies. *Leucocytozoon* is transmitted by black flies of the family simulidae but not all species of black flies are natural vectors, while many species of the member have been involved as probable vectors are (*Simulium* spp.) (AL-Zurfi, 2015). *Haemoproteus* is the most common blood parasites of birds and it is transmitted by blood sucking insects like mosquitoes, biting midges (*Culicoides*), louse flies (*Hippoboscidae*) and *Tabanid* flies (*Tabanidae*)(Ausraful *et al*., 2013). *Plasmodium* is the protozoal disease of chickens which is transmitted with the vectors *Mosquitoes*, *Hippoboscide* flies, black flies, mites, ticks and fleas can transmit the infective stage ***(***Adriano and Coerdeiro, 2001). *Aegyptianella pullorum* is the only species which infects poultry with soft ticks of fowl (*Argas persicus*) from infected to non-infected hosts (Tarello, 2005).

2.5 Life cycle and Development of Haemoparasites

The life cycle of *Plasmodium*, *Haemoproteus* and *Leucocytozoon* are determined by the insect vectors and a host avium. All have similar lifecycle, however, they differ in important aspects. By considering

this differences in vectors, life cycles and epidemiology of the organisms the presence of both erythrocytic chizonts and gametocytes in infections with *Plasmodium* is a key difference from *Haemoproteus* and *Leucocytozoon*, these two latter undergo schizogony only in fixed non-circulating cells in the host (Valkiūnas *et al*., 2005).The vector insects inserting infective sporozoites along with saliva to the host, then sporozoites invade tissues and reproduce scizonts to produce multiple merozoites. Then, merozoites penetrate red blood cells and develop into infectious gametocytes. The second new vector feeds on birds and becomes infected. Gametocytes mature, and undergo sexual reproduction in midgut .Oocysts become encapsulated on the outer midgut wall. The oocysts rupture and sporozoites invade salivary glands (Friend and Franson, 2001).

2.5.1 Life Cycle of *Plasmodium* spp.

An infected vector mosquitoes bites an uninfected chicken, infective sporozoites are passed in to the chicken blood and via the blood stream reaches to the liver; in the liver the sporozoites develop in to pre-erythrocytic schizonts which then become merozoites; merozoites enter erythrocytes and develop in to macrogametocytes and microgametocytes. Schizonts multiply in red blood cells (Valkiunas, 2005).

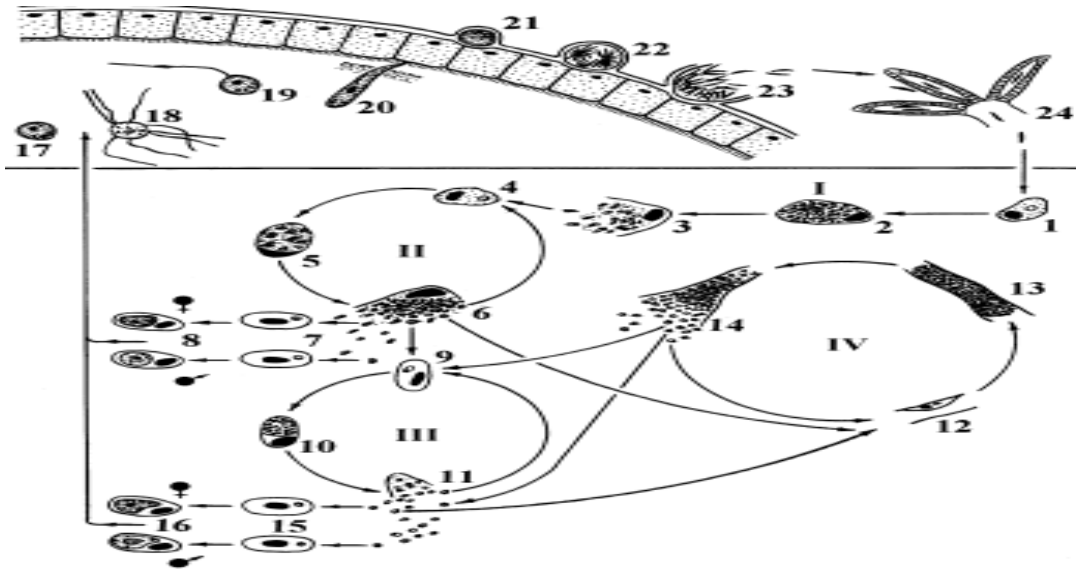

Figure 1: Diagrammatic Representation of Life Cycle of *Plasmodium* spp.

Upper part, in vector, lower part in chicken:I,II – primary exoerythrocytic merogony, III erythrocytic merogony, IV – secondary erythrocytic merogony;1 - sporozoites in reticuloendothelial cell;2, 3 – cryptozoites, 4 – merozoites in macrophages;5, 6 – metacryptozoites; 7 – merozoites in erythrocytes;

8 – gametocytes, 9– merozoites in erythrocytes, 10,11– erythrocytic meronts;12 – merozoites in endothelial cells of capillaries; 13,14–phanerozoites;15–merozoites in erythrocytes;16 – gametocytes;17– macrogamete; 18 – exflagellation of microgametes; 19 – fertilization of macrogamete, 20 –ookinete penetrating the peritrophic membrane;21– young oocyst;22, 23 – sporogony, 24 –sporozoites in the salivary glands of vector. Source: (Valkiunas, 2005).

2.5.2 Life Cycle of *Haemoproteus* spp.

A vector midge or *Hippoboscid* ingests gametocytes in red blood cells of an infected chicken; inside the insect vector the parasites migrate from the insects gastrointestinal tract to the blood stream, then to the salivary glands as sporozoites; sporozoites are injected in to the blood stream of a new chicken host when the insect feeds. Sporozoites migrate from chicken blood stream into endothelial cells of lung, liver, bone marrow and spleen; where they develop into schizonts; each schizonts contains many merozoites that are released into bloodstream when the endothelial cells dies; merozoites in the bloodstream enter to RBC's and become gametocytes. Gametocytes in chicken RBC's can become infective for 7 days after they enter to chicken RBC's (Valkiunas, 2005).

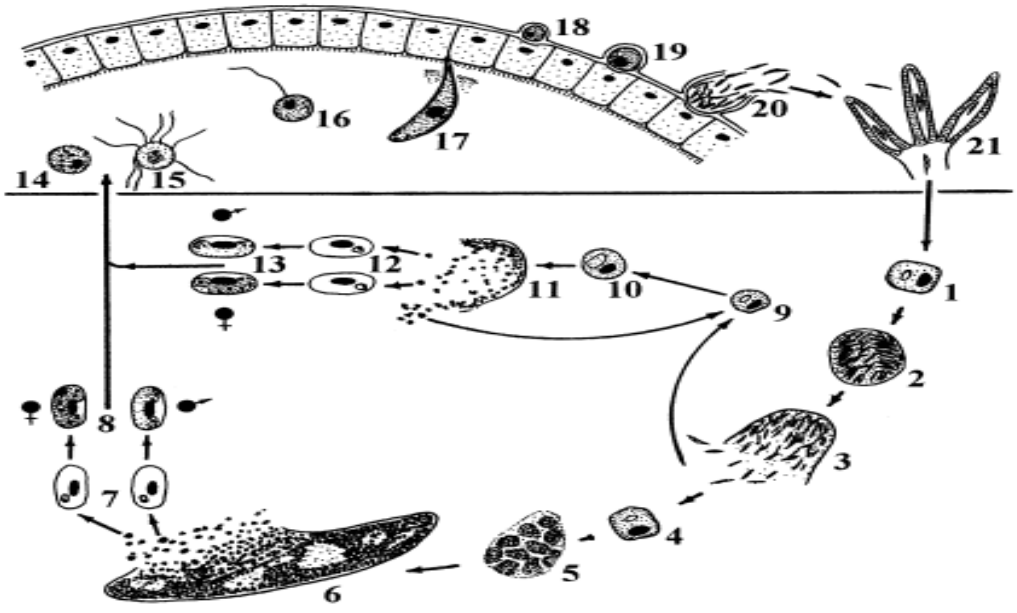

Figure 2: Diagrammatic Representation of Life Cycle of *Haemoproteus* spp.

Upper part, in vector; lower part in, chicken:1–sporozoites in endothelial cells;2, 3– exoerythrocytic meronts of the first generation with elongated merozoites; 4–merozoite in endothelial cell;5, 6 – growing and mature megalomeronts in skeletal muscles in respectively; 7 –merozoites in erythrocytes; 8- mature gametocytes;9–merozoite in reticuloendothelial cells of spleen;10,11–growing and mature meronts in spleen respectively;12 –merozoites in erythrocytes; 13 –mature gametocytes;14 – macrogamete;15 –exflagellation of microgametes;16 –fertilization of macrogamete;17 –ookinete penetrating the peritrophic membrane; 18 –young oocyst; 19, 20 – sporogony; 21 –sporozoites in the salivary glands of the insect vector. Source: (Valkiunas, 2005)

2.5.3 Life Cycle of *Leucocytozoon* spp.

The infected blood containing gametocytes ingested by the vector, the gametocytes develop into sporozoites inside the fly, the fly injects the sporozoites into the bloodstream of the new host. Sporozoites travel from the bloodstream to invade endothelial and parenchymal cells of various organs such as liver, heart and kidney. Sporozoites develop into schizonts, which then rupture and release merozoites that invade RBC's and leucocytes (Valkiunas, 2005).

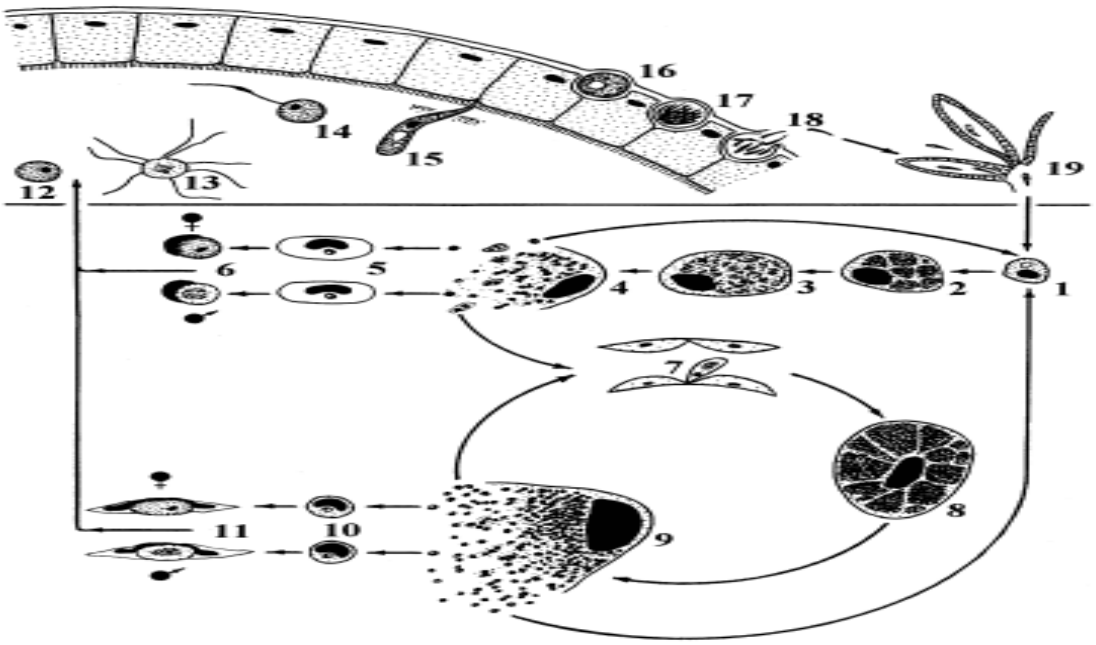

Figure 3: Diagrammatic Representation of the Life Cycle of *Leucocytozoon* spp.

Upper part, in vector; lower part in chicken:1-sporozoites or merozoite in hepatocyte;2-4 hepatic meronts;5 -merozoites in erythrocytes;6-gametocytes;7-syncytium(fragment of hepatic meronts with

two or more nuclei) or merozoites in reticuloendothelial cell;8,9 –megalomeronts;10 –merozoites in mononuclear leukocytes;11 –gametocytes in fusiform host cells; 12 –macrogamete; 13 –exflagellation of microgametes;14 –fertilization of macrogamete;15 –ookinete penetrating the peritrophic membrane;16 –young oocyst;17, 18 –sporogony;19 – sporozoites in the salivary glands of the vector. Source: (Valkiunas, 2005)

2.5.4 Life Cycle of *Trypanosoma* spp

The life cycle of avian *Trypanosoma* is similar to other animals. During a blood meal on the bird host, an infected insect vector injects metacyclic trypomastigotes into skin tissue. The parasites enter in to the lymphatic system and pass into the bloodstream. Inside the host, they transform into bloodstream trypomastigotes, are carried to other sites throughout the body, reach other blood fluids (e.g., lymph, spinal fluid), and continue the replication by binary fission. The entire life cycle of *Trypanosoma* is represented by extracellular stages. The insect vectors become infected with bloodstream trypomastigotes when taking a blood meal on an infected host. In the insects' midgut, the parasites transform into procyclic trypomastigotes, multiply by binary fission, leave the midgut, and transform into epimastigotes. The epimastigotes reach the fly's salivary glands and continue multiplication by binary fission (Tarello, 2005 and Murray *et al*., 1983).

2.5.5 Life Cycle of *Aegyptienella* spp.

The single inclusion body of the primary stage of the life cycle of *Aegyptienella*, after it has been phagocytosed by erythrocytes.The maturity of the individual body, through asexual reproduction, the vacuolar membrane is ruptured and the organisms are released, thereafter each parasites penetrates the uninfected red blood cells as the primary or immature inclusion body, developed until divided into multiple parasites as secondary or mature inclusion bodies (Sells *et al*., 1976). The developmental cycle of *Aegyptienella* consists of the formation of initial bodies, developmental forms and marginal bodies. Following feeding by an adult tick on an infected chicken, 25 days or more is required before the organism is transmit to another uninfected chicken (Sabuni *et al*., 2011).

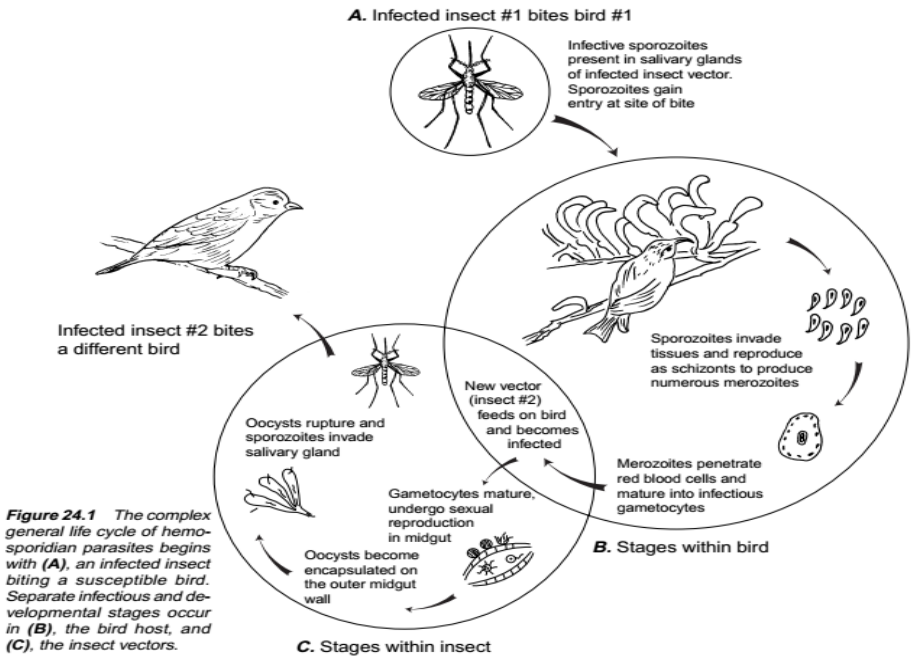

Figure 4: General Developmental Stage of Haemosporidian Parasites

Source: (Friend and Franson, 2001)

2.6 Pathogenesis and Pathogenicity

Chickens, once contract the infection with haemoparasites, they remain infected throughout the life time. Avian chronic infection caused by haemoparasite induces reproductive costs (Marzal *et al*., 2011). Haemoparasites have important implications for the avian fauna and their conservation as well as life history (Merino *et al*., 2000). Haemoparasite infections can cause serious compromised host fitness, myopathy, myositis, myonecrosis as well as mortality in chicken and the true flying birds (Klein *et al*., 2004). Clinically the disease is manifested with anemia due to erythrocytic parasitism mostly in the immunocompromised hosts (Cardona *et al*., 2002). The typical pathology of infection with these parasites includes anaemia, enlargement of the liver and spleen. Microscopically, there is an ischemic necrosis and associated inflammation in the heart, brain, spleen and liver due to occlusion of blood vessels by megaloschizonts in endothelial cells. Ruptured schizonts may induce granulomatous reactions in the surrounding tissues (Cardona *et al*., 2002).

The excess mortality due to *Leucocytozoon* in adult chicken appears to occur as a result of debilitation and increased susceptibility to secondary infection (Wlliam, 2005). Pathogenesis of *Leucocytozoon* appears post five days infection, many schizonts develop in hepatocytes leading the cell to rupture. Post seven days infection megaloschizonts begin to appear in spleen, lymph node and other tissues. Gametocytes accumulate in liver after 12 days infection haemorrhagic scars produces by rupturing megaloschizonts in area of infection. Infection with *L. caulleryi* in chickens has a tropism to the reproductive tract and it is associated with oviduct inflammation, edema and decreased egg production. Peritoneal, perirenal, and subdural haemorrhages are reported with severe diseases caused by *Leucocytozoon* (Ferrel *et al*., 2007). The pathogenic impact of haemosporidioses on their hosts is enormously complicated and varied, which mostly determined both by their complex life cycles and the complicated epidemiology of the diseases. Pathological changes in poultry caused by certain species of haemosporidians are different (Wettere and Arnauld, 2013).

2.6.1 Tissue Stage

A characteristic feature of the development of first generation exoerythrocytic meronts, which induced by sporozoites, is that they do not cause serious pathology in infected poultry regardless of the group of haemosporidians. The number of meronts is not large; their size is very small; they rapidly develop and the inflammatory reaction is not pronounced. An exception are the first generation exoerythrocytic meronts in *Haemoproteus*, which cause the necrosis of adjacent muscle fibers (Atkinson *et al*., 2008). If the infection with *Leucocytozoon* species is substantial, plentiful meronts of the first generation can cause distending and obstruction of liver sinosids. (Valkiunas, 2005).

In the case of *Leucocytozoon*, the pathological changes are mostly associated with the development of megalomeronts in the spleen, liver, lungs, heart and brain. Mature megalomeronts reaches up to 200µm in diameter and they are enclosed by a fibrous capsule like wall which contain many thousands of merozoites. A clearly expressed inflammatory reaction is usually observed around the megalomeronts. Infiltrates frequently contain erythrocytes, macrophage, plasmatic cells and hetrophils. After the termination of merogony, the capillaries adjacent to the parasite and megalomeronts become burst then, hemorrhage is pronounced. If the parasite is develops in the brain indications of cerebral paralysis are found. The disease caused by *L. caulleryi* in domestic chicken in South East Asia is often called Bangkok haemorrhagic disease. During the final stage of the merogony,

necrotic centers are formed in place of ruptured megalomeronts and calcificates are found (Parker *et al*., 2006).

The most sever caused by the *Plasmodium* species is related with the blockage of brain capillaries and other vital organ capillaries. As the result of this, the blood supply of the affected organs is disturbed, the tissues surrounding the meronts suffer from anoxia, and the cell become die off. Necrosis of the tissues adjacent to the meronts is substantial (Palinauskas *et al*., 2011). In cases of severe *Plasmodium* and *Haemoproteus* infections, excessive amount of insoluble pigment is accumulated in the macrophages of the spleen and liver, and these organs acquire a black hue(shade) (Atkinson *et al*., 2008).

2.6.2 Blood Stage

The most serious pathological consequences of the development of haemosporidians in the blood is the destruction of red blood cells. The general causes of anemia chicken affected by haemosporidioses is the active removal of infected erythrocytes from the blood circulation by the cells of reticuloendothelial system in the spleen, liver and bone marrow. Acute anemia is developed in those cases, when the process of erythropoiesis and introduction of erythroblast cells in the blood do not compensate the losses of erythrocytes. Destruction of erythrocytes during the development of *Plasmodium* species is also associated with the development of many erythrocytic meronts (Atkinson *et al*., 2008)

During the *Leucocytozoon* infection, anemia is intensified because of the destruction of uninfected erythrocytes due to the appearance of anti-erythrocytic factor in the blood plasma. The other peculiarity *Leucocytozoon* infection is serious pathological changes may induce gametocytes circulating in the blood, which form large host-parasite complex together with the infected cells reaches up to the length of 40μm (Valkiunas, 2005).

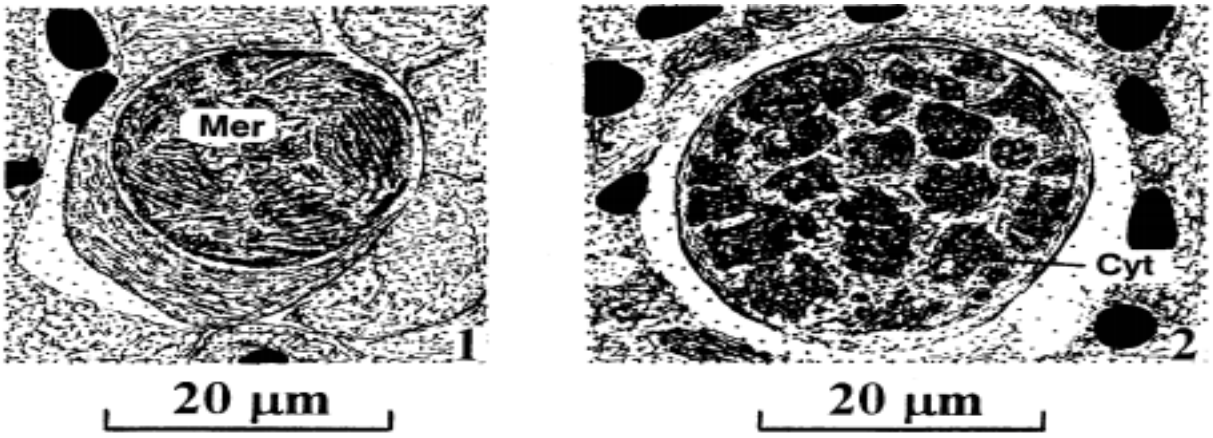

Figure 5: Exoerythrocytic Meronts of Haemoproteus from the Muscle of Poultry

Source: (Atkinson *et al*., 2008)

2.7 Clinical Findings

Chickens with acute infections of *Plasmodium*, *Haemoproteus* and *Leucocytozoon* may display similar signs. In the infected chicken, the clinical disease is related with fever, depression, anorexia, loss of body weight, dyspnea, ocular haemorrhages, haemolytic anaemia, loss of appetite, emaciation, listlessness, difficulty in breathing, weakness and lameness in one or both legs (William, 2005 and Ferrel *et al*., 2007). Mortality in chicken due the disease may be up to 90 %; severe infections by *Haemosporidian* can lead to death and involves different physiopathological phenomena such as anemia, thrombocytopenia and inflammation (Cannell *et al*., 2013). Avian Haemosporidioses can be severe or even fatal for domestic birds and for birds in zoos (Ferrell *et al*., 2007). At the population level, haemoparasites can affect their hosts by reducing fitness parameters such as body condition, reproductive success and survival. *Haemosporidians* are known to be highly pathogenic to domestic chicken with high mortalities (Hasson, 2015).

Aegyptienella pullorum is the only spp. which can cause high morbidity and mortality in domestic and wild chicken. The clinical findings are dyspnea, arthritis, sinusitis, reduced speed and strength in flight, poor appetite, weight loss, weakness, anorexia, vomiting, bumble foot, white diarrhea, blood mixed droopings and tick infestation (Tarello, 2005).

2.8 Investigation of Haemoparasites

Identification of haemoparasite is mainly depend on examination of blood films, organ impression smears, concentration methods, histological sectioning of the organs and tissues and molecular based techniques (Valkiunas *et al*., 2011).

2.8.1 Classical Techniques

Extracting blood directly from a vein with hypodermic needle and syringe with appropriate gauge needle or by using a pricker. Blood is withdraw from the brachial vein (wing vein) or jugular vein collected in universal bottles containing EDTA to prevent clotting. The prepared blood films allow to air-dry within 5–10 seconds, then fixed with methanol for 5 minutes, stains with 10% Giemsa for 15 minutes, wash with tap water, blot and examine under the microscope with higher magnification of (X 1000) oil immersion. The haemoparasites detected and identified according to Soulsby (1982; Valkiûnas, 2005and Sabuni *et al*., 2011).

Red blood cells which are affected by *Leucocytozoon* becomes enlarged and elongated and forms a pair of horn-like extensions at any end of the cell. *Plasmodium* and *Haemoproteus* produce fewer changes in their host's red blood cells, nevertheless these parasites may cause enlargement of infected host cells and displacement of the red blood cell nucleus to one side (Soulsby, 1982). Finding of a *Plasmodium* infection is dependent on detecting the presence of asexually reproducing stages of schizonts and gametocyte stages in the red blood cells of the infected chicken **(**Friend and Franson, 2001). Analysis of avian *Trypanosoma*, blood trypomastigotes are easily found in thin blood films (Murray *et al*., 1983).

2.8.2 Preparation of Impression Smears from Organs and Tissues

The impression smears are made for the investigation of the tissue stages of haemosporidians. A small piece of an organ reaches $1cm^3$ is removed with a scalpel or razer then blotted gently with filter paper to remove excess blood from the cut sample. The prepared piece of tissue is pressed on the clean glass slide to left over the imprints on the slide. The prepared imprint smears become air dried, fixed, stained with giemsa stain and examined as the same way as the blood smears (Valkiûnas, 2005).

2.8.3 Concentration Methods

For the revealing of haemoparasites, sufficient amount of life blood volume is required. The detection of chicken haemoparasites might be increased by centrifugation of blood in a capillary tube and subsequent light microscopic examination of the serum immediately above the buffy-coat layer of *Trypanosoma* species and stained, thin film prepared from the buffy-coat for the *Haemoproteus* species and *Leucocytozoon* species (Murray *et al*., 1983).

2.8.4 Histological Sectioning of the Organs and Tissues

The preparations of tissue stages of *Haemosporidians* of high quality are made when histological methods are used. It is recommended to make histological preparations from the materials selected by the examination of impression smears to detect the primary tissue stages (Valkiûnas, 2005.

2.8.5 Molecular Biology Method

Furthermost, outstandingly the polymerase chain reaction (PCR) have been applied for the detection of chicken haemoparasites. PCR provides an effective way to detect haemoparasites when only small volume of blood are available as well as few haemoparasites are present. Use of multiplexed PCR for the concurrent detection of different haemoparasite encourages convenient investigation of samples (Sehgal *et al*., 2006 and Parker *et al*., 2006).

2.9 Prevention and Treatment

2.9.1 Vector control

Prevention of haemosporidioses is based on the isolation of birds from the vectors whose period of activity is associated with the warm season of the year. During the period favorable for infection, small groups of birds and an individual expensive specimens should be kept indoors put in cages covered with fine-mesh bolting silk which prevents blood sucking dipteran insects from penetrating there. Newly introduced chickens are treated in the same way, as well as the birds returning from the exhibitions, breeding and markets (Wettere and Arnauld, 2013). Prevention of the avian hemoparasite is reliant on reducing transmission from infected birds to healthy birds through reduction or elimination of vector populations. Most techniques rely on habitat management to reduce vector

breeding sites or depend on the application of pesticides that affect larval or adult to reduce populations (Friend and Franson, 2001).

Prevention of haemosporidioses in industrial poultry farming is a difficult problem. One should kept in mind is that young birds are more susceptible; they experience the illness harder perish more often (Soulsby, 1982). To minimize the risk of transmiting ectoparasites from wild birds to domestic, fencing is the crucial important in order to keep other birds away. The nymphal and adult stages of fowl ticks feed on their hosts for a limited period, therefore, the control of ticks requires treatment of the environment in which the chickens found (William, 1995).

2.9.2 Chemotherapy and Chemoprophylaxis

Treatment and prevention of Leucocytozoonosis

Bringing of preventive compounds decrease chicken damage caused by leucocytozoonosis, Clopidol in a dose of 0.025% is added to the food during the period favorable for the transmission of the parasite this effectively controls the parasitemia. A good result in prevention from *L. caulleryi* is obtained if Pyrimethamine in dose 0.00005 to 0.0001 % or sulphadimethoxine in dose 0.005% is added to food, or their combination is used in doses of 0.0001 and 0.001% (Soulsby, 1982).

Treatment and prevention of Haemoproteosis

In case of haemoproteosis, it is suggested to put on Quinacrine hydrochloride or chloroquine phosphate, 100 mg should be dissolved with 113 ml of drinking water and give for chickens every day during 7-14 days, repeating the treatment after two weeks. In addition, 250mg Quinacrine hydrochloride is dissolved in 113 ml of drinking water and give to chickens daily for as long as 30 days. Atebrine and plasmochine is used to reduce the parasitemia of *haemoproteus* species, but have no effects on the erythrocytic meronts stages (Tarello, 2005).

Treatment and prevention of Plasmodium

Copious preparations are tested to treat chicken malaria. Chloroquine phosphate in doses of (5 mg per 1 kg), Paludrine (7.5 mg per 1 kg), and Pyrimethamine (0.3 mg per 1 kg) are effective against *P. gallinaceum*. The dose of premaquine phosphate is 0.003 mg per kg daily for the period of three days.

The first dose of chloroquine phosphate introduced together with premaquine phosphate is 10 mg. After 6, 8 and 24 hours, the intubation of chloroquine phosphate is repeated at a dose of 5mg/kg (Tarello, 2005 and Wettere and Arnauld, 2013).

Table 2: Drug of Choices to Treat Chickens Infected with Haemoparasite

Haemoparasites	**Drug of choice**
	Chloroquine phosphate
	Premaquine phosphate
	Pyrimethamine- sulphadoxine
Haemoproteus spp.	Mefloquine
	Sulfamonomethoxine
	Sulfachloroptrazine
	Doxycycline
	Halofuginone
	Atovaquone-proguanil combination
Aegyptynella spp.	Doxycycline
	Pyrimethamine
	Qyrimethamine- sulfamonomethoxine
Leucocytozoon spp.	Clopidol, Atebrine
	Trimethoprim-sulfamethoxazole
	Melarsomine & primaquine.
Plasmodium spp.	Chloroquine phosphate
	Premaquine phosphate
Trpanosoma spp.	Melarsomine (Cymelarsan)

Source: (Tarello, 2005 and Wettere and Arnauld, 2013)

2.9.3 Flock structure

The susceptibility and occurrence of parasitic diseases vary between different age groups of chicken. Older chickens may be carriers of a range of haemoparasites without showing clinical signs. As a result, it may be beneficial to separate different age groups vis-a-vis the ‘all in- all-out’ principles (Halima, 2007).

3. MATERIALS AND METHODS

3.1 Study Areas

The study was conducted in three selected districts of Mecha (mid highland) Bahirdar-Zuria and South Achefer (midland) in West Gojjam zone, Amhara Region, having different agro ecologic conditions from October 2016- May 2017. West Gojjam is located on North West part of the country. Its latitudinal and longitudinal extension is $11^{0}25^{'}$N – $11^{0}55^{'}$N and $37^{0}.04^{'}$E – $37^{0}.39E^{'}$ an altitude of 1000-3000. The agroecology of the study area is 15% highland, 82% midland, and 3% lowland. It has an annual rainfall 200-1200 mm Hg and the average temperature ranges from 17^{o}- 27^{o}c (CSA, 2016). The mean annual temperature ranges from 22-27^{o}c in the lowlands and between 10 and 22^{o}c in the highlands up to 3,000 meters above sea level. West Gojjam is bordered on the south by Abay River and on the north by Lake Tana. Its highest point is mount Amedamit (Adama). It has a population of cattle, 2,319,049, sheep, 1,206,147, goats, 355,190, equines, 389,225, beehives, 197,222. The poultry population is comprised of 3.3 million indigenous, 0.11million cross and 0.043 million exotic breeds (CSA, 2016). Local, cross and exotic breeds of poultry are raised in the areas which are managed under extensive, semi-intensive and intensive farming system, respectively (WGARDO, 2008).

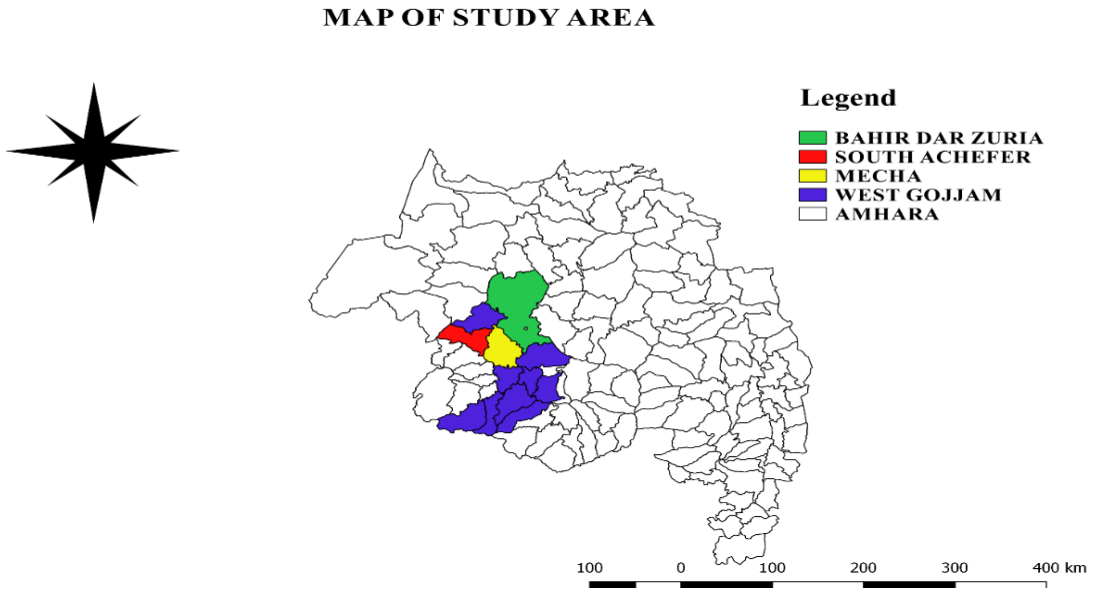

Figure 6: Map of the Study Areas

3.2 Study Animals

Three hundred and eighty four chickens comprising of cross (n= 46) and local (n= 338) breeds of scavenging domestic chickens were used for the purpose of this study. Both sexes females (n=257) and males (n=127) and different age groups of chickens were included in the study from three districts with different agroecological conditions. The chickens were usually brought from the nearby villages where they were raised by the free-range forager system. The ages were determined subjectively based on the size of crown, length of spur and flexibility of the xiphoid cartilage together with information from the farmers. The chickens were classified as adults (cock or hen), growers (pullet or cockerel) and chicks (male and female).The chickens were categorized into three age groups as chicks (aged < 24 weeks), growers (24 to 48 weeks) and adults (aged > 48 weeks) according to Maina (2005).

3.3 Study Design and Sample Size Determination

A cross sectional study design was conducted from October 2016-May 2017 to determine the prevalence and type of hemoparasites, to measure the hematological parameters and the risk factors (sex, age, breed, and agroecology and management system) associated with haemoparasite infection

in village chickens in the study area. In addition, a questionnaire survey was conducted to assess the control practices of different poultry diseases, management systems and level of awareness of the poultry owners about poultry diseases in the study areas. The sample size was determined based on the assumption of possible or expected prevalence rate of the disease recorded in the study area. Since there was no previous study conducted in the study area, the expected prevalence was taken as 50%. The sample size was calculated by the formula of Thrusfield (2005), with 95% confidence interval and 5% absolute precision. Therefore, the maximum required sample size was 384.

According to the formula, $n = z^2 * \frac{(pexp * q)}{d^2}$

n= the required sample size

Pexp= expected prevalence

$q = (1-p_{exp})$

d= Desired absolute precision (5%)

Where Z (a multiplier for 95% confidence interval)

Z= 1.96; p= 50% and d= 5%. Therefore, the maximum required sample size was 384.

3.4 Sampling Method

Systematic simple random sampling methods were used to select the study animals with taking the n^{th} position. Two peasant associations were selected from each district purposive method based on their agroecological representativeness (midland and highland) and relative ease of accessibility of transportation.

3.5 Methods of Data Collection

3.5.1 Questionnaire Survey

The semi structured questionnaire survey was conducted by interviewing a total of one hundred eight chicken owners in the study area of the selected three districts of each, two PA's about the management system of (feed preparation, shelter of chicken and rearing system), purpose of rising the chicken, number of chicken owned, type of chicken breeds, commonly observing diseases and their clinical symptoms, medication when become sick and the commonly administered drugs. The sample size of the respondent was calculated by the formula of Arsham (2005).

$N=0.25/SE^2 * 100$; where SE=b/n 0.1 & 0.5

3.5.2 Haemoparasite Examination

Collection of blood sample

For parasitological examination and hematology analysis, two milliliters (2ml) of blood sample were withdrawn by venipuncture directly from the wing vein of each of the selected birds, using sterile syringes and needles of 23 or 25 gauge and put into a sterile Ethylene Diamine Tetra Acetic Acid (EDTA) coated vacutainer tubes. During blood sample collection, each chicken was fixed on its side or back, the wings were stretched and feathers were plucked to expose the brachial vein (wing vein) and the area was disinfected using a cotton swab contained in 70% alcohol (Campbell, 1988) (Annex 1). The samples collected were then gently shacked with the anticoagulant to prevent clotting and later transported to the laboratory for further investigations and analysis.

Laboratory procedures

Thin blood technique was carried out according to the standard procedures of (Thrall, 2004). Thin blood smears from each sample were prepared with drops of blood placed at the end of grease free glass slides and made thin with a spreader. The preparations were allowed to air-dry completely and fixed with methanol for three minutes. The films were then placed on the staining rack and covered with 10% Giemsa stain and allowed to stand for about 30 minutes. After this, the slides were flooded with tape water to dilute the stain making sure that it did not overflow and allowed to stay for 10 minutes. Giemsa-stained thin blood smears were screened for haemoparasites microscopically using the x100 oil immersion objective (Thrall, 2004). The haemoparasites detected were identified by the morphology of parasitic developmental stage in intracellular and extracellular position according to the methods described by Soulsby (1982) and Campbell (1988) (Annex 2 & 3).

3.5.3 Determination of Packed Cell Volume (PCV)

Determination of packed cell volume of chicken was conducted by using automated Hem Analyzer. The normal range of packed cell volume (PCV) value of domestic chicken is 30-49 %. If the result becomes below the average, the chicken becomes anemic (Campbell, 1988) (Annex 4).

3.5.4 Total Red Blood Cell count (TRBC)

The total red blood cell count of chicken was processed by Automated Hem Analyzer. The normal range of red blood cell count of domestic chicken is 2.5-3.9× 10^{12}/L (Campbell, 1988) (Annex 4).

3.5.5 Total White Blood Cell count (TWBC)

The total white blood cell (TWBC) count was conducted by using Automated Hem Analyzer. The normal average WBC count of domestic chicken is $1.9\text{-}9.5\times10^3$/L (Campbell, 1988) (Annex 4).

3.5.6 Estimation of Mean Cell Volume (MCV)

The MCV of chicken was analyzed with Automated Hem Analyzer. The normal range MCV of each red blood cell of domestic chicken is 104-135 fl (Campbell, 1988) (Annex 4).

3.5.7 Estimation of Mean Corpuscular Hem (MCH)

The average amount of hemoglobin per red blood cell. The red blood cell space of chickens were measured by Automated Hem Analyzer. The normal range of MCH of chicken is 32-43.9 pg (Campbell, 1988) (Annex 4).

3.5.8 Estimation of Mean Corpuscular Hemoglobin Concentration (MCHC)

The average concentration of hemoglobin in the red blood cell (MCHC) was measured with Automated Hem Analyzer. The normal range of MCHC of domestic chicken is 30.2-36.2 dl (Campbell, 1988) (Annex 4).

3.5.9 Estimation of Level of Hemoglobin (Hb)

Measures the amount of hemoglobin per red blood cell and the blood ability to carry out oxygen. In chicken species estimation of hemoglobin is hindered by the presence of nuclei in the red blood cells. Level of hemoglobin estimation relies on colorimetric measurement of hemoglobin released after the lysing of the RBCs. Hemoglobin level was measured by Automated Hem Analyzer. The normal Hb level of domestic chicken ranges from 10.2- 15.7 g/dl (Campbell, 1988) (Annex 4).

3.6 Data Management and Analysis

The collected data were entered in to Microsoft Excel spread sheet and analyzed by STATA soft-ware vir.12.0. The questionnaire survey was described by frequencies and the haemoparasite infection prevalence was estimated by dividing the number of haemoparasite infected chickens by the total

number of examined chickens. Univariable logistic regression analysis were used to associate the haemoparasite infection with different risk factors. Chi-square was also applied for data analysis to determine the association between prevalence of haemoparasite infection with different risk factors. Moreover, independent t-test was used to compare the mean hematology findings of the chicken's infected with haemoparasite from non-infected.

4. RESULTS

4.1 Over all Prevalence of Chicken Haemoparasites

A total of 384 village chickens of different age and sex groups, from two agro ecological zones were examined for the presence of haemoparasites. Out of 384 chickens examined, 71 (18.5%) were infected with haemoparasites. Four species of haemoparasites were found during this study. These were *Aegyptienella, Haemoparoteus, Leucocytozoon* and *Plasmodium. Aegyptienella* spp. was the most prevalent haemoparasite (40.8%) followed by *Haemoproteus* (15.5%), *Leucocytozoon* (12.7%) and *Plasmodium* spp. (4.2%) in single form of infections. In chickens with mixed infections, the highest record was in *Aegyptienella* and *Haemoproteus* (8.5 %) followed by *Plasmodium* and *Aegyptienella* (7.0%), *Leucocytozoon* and *Plasmodium* (4.2 %), *Haemoproteus* and *Plasmodium* (2.8 %); *Aegyptienella, Plasmodium* and *Haemoproteus* (2.8%) also *Aegyptienella, Plasmodium* and *Leucocytozoon* was (1.4) % in triple form of infections (Table 3).

Aegyptienella spp.

The inclusion bodies of *Aegyptienella* spp. was present inside and outside of the red blood cells. The organism was looks like round inclusion bodies and was identified inside and outside the cytoplasm of the red blood cells membrane, since erythrocytic vesiculation is the mode of entrance or exit of initial bodies from the red blood cells (Figure 7).

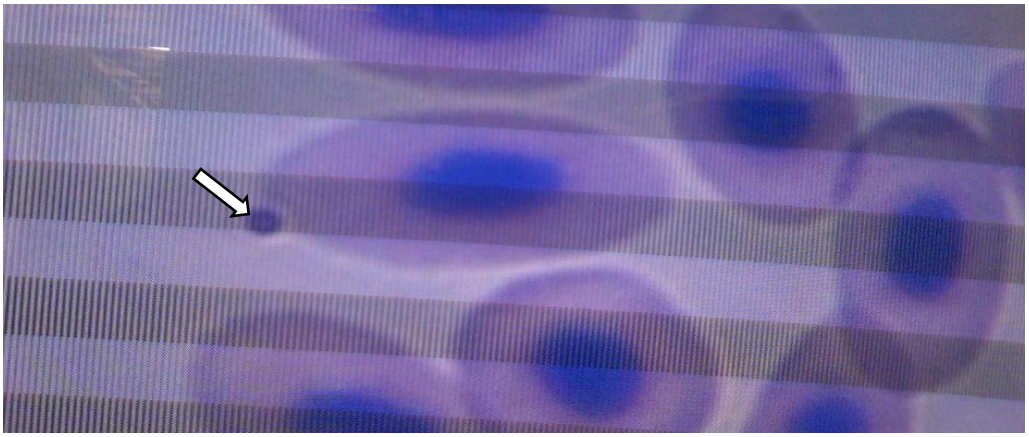

Figure 7: *Aegyptienella* spp. (arrow) Infected RBC in the Thin Blood Film of Chicken stained with Giemsa's stain (X 100)

Haemoproteus spp.

The identified gametocyte stage was presented within cytoplasm of erythrocytes. The gametocyte was very small granule like in structure stained somewhat pinkish dotes (Figure 8).

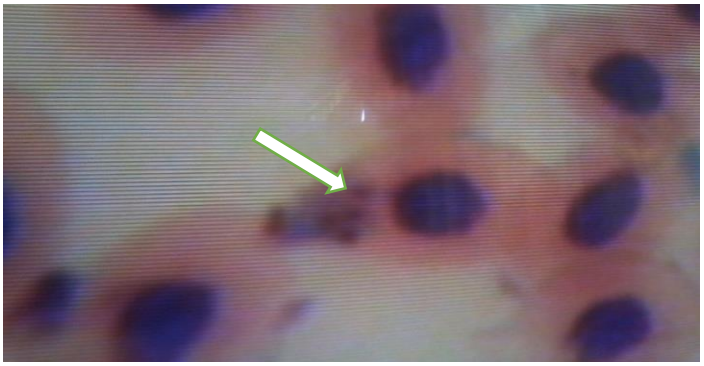

Figure 8: *Haemoproteus* spp. (arrow) Infected RBC in the Thin Blood Film of Chicken stained with Giemsa's stain (×100)

Leucocytozoon spp.

The gametocyte, which was identified from the periphery of RBC were elongated (Figure 9).

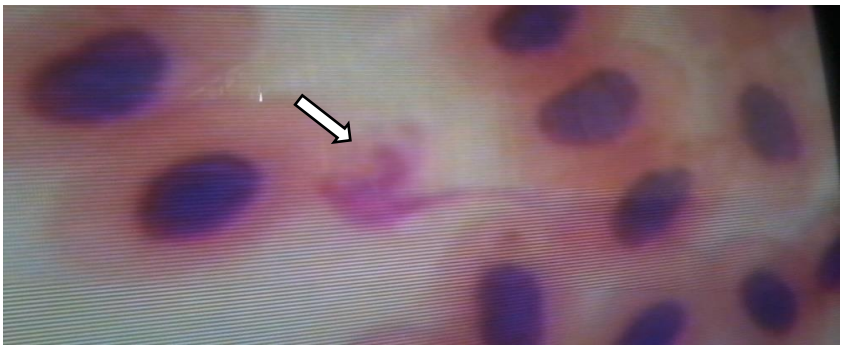

Figure 9: *Leucocytozoon* spp. (arrow) Infected RBC in the Thin Blood Film of Chicken stained with Giemsa's stain (X 100)

Plasmodium spp.

The identified merozoite stage of plasmodium was present in the red blood cell cytoplasm. The merozoite was signet ring shaped in structure (Figure 10).

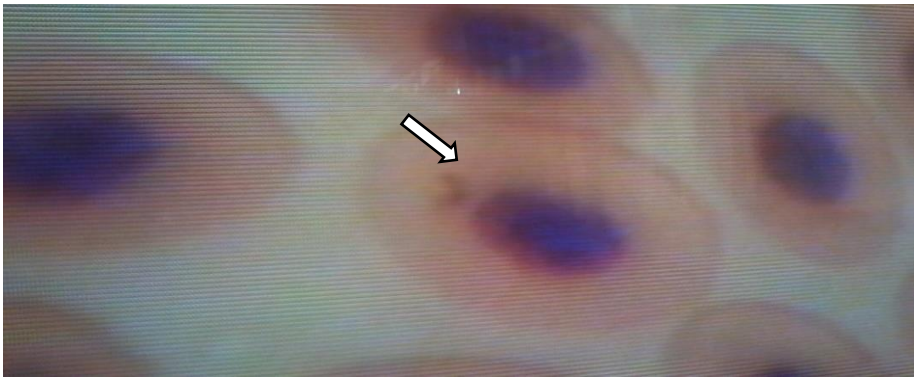

Figure 10: *Plasmodium* spp. (arrow) Infected RBC in the Thin Blood film of chicken stained with Giemsa's stain (X100)

Table 3: Type of Identified Haemoparasites and their Prevalence

Type of hamoparasites	**Number of infected chicken**	**Prevalence %**
Aegyptienella spp.	29	7.6
Haemoproteus spp.	11	2.9
Leucocytozoon spp.	9	2.3
Plasmodium spp.	3	0.8
Aegyptienella and Plasmodium spp.	5	1.3
Leucocytozoon and Plasmodium spp.	3	0.8
Aegyptienella and Haemoproteus spp.	6	1.6
Haemoproteus and Plasmodium spp.	2	0.5
Aegyptienella, Plasmodium and Leucocytozoon spp.	1	0.2
Aegyptienella, Plasmodium and Haemoproteus spp.	2	0.5
Overall prevalence	71	18.5

4.2 Prevalence of Chicken Haemoparasites in Relation to Different Age Groups

Out of the 384 examined domestic chicken, 18.5% were positive of haemoparasite in a single and mixed infections. Growers were more infected than adults and chicks with prevalence of 24%, 18.9 % and 2.4 % in a single form of infection, for the meantime, 7.1%, 1.9% and 0% record was observed in mixed form of infections in adult, growers and chicks, respectively. The association among age group was statistically significant (x^2=13.36; P<0.05).The occurrence of chicken haemoparasite infection in grower chickens were 0.93 times more likely to be affected by haemoparasite infection

than in chicks (OR; 0.93, 95% CI= 1.27-72.8); meanwhile, the occurrence of chicken haemoparasites in adults were 0.66 times more likely to be infected than the occurrence of the infection in chicks (OR; 0.66, 95% CI= 0.37-1.2) (Table 4 and Table 11).

Table 4: Prevalence of Chicken Haemoparasites in Relation to Different Age Groups

Haemoparasites identified	**Age**			**Chi^2**	**p-value**
	Chicks (n=42)	**Growers (n=104)**	**Adults (n=238)**		
Aegyptienella spp.	0(0.0%)	12(11.5%)	17(7.1%)		
Haemoproteus spp.	0 (0.0%)	4(3.8%)	7(2.9%)		
Leucocytozoon spp.	1(2.4%)	5(4.8%)	3(1.3%)		
Plasmodium spp.	0(0.0%)	2(1.9%)	1(0.4%)		
Aegyptienella and Plasmodium spp.	0(0.0%)	2(1.9%)	4(1.7%)	13.36	0.009*
Leucocytozoon and Plasmodium spp.	0(0.0%)	0(0.0%)	3(1.3%)		
Aegyptienella and Haemoproteus spp.	0(0.0%)	0(0.0%)	2(0.8%)		
Haemoproteus and Plasmodium spp.	0(0.0%)	0(0.0%)	5(2.1%)		
Aegyptienella, Plasmodium and Leucocytozoon	0(0.0%)	0(0.0%)	2(0.8%)		
Aegyptienella, Plasmodium and Haemoproteus	0(0.0%)	0(0.0%)	1(0.4%)		
Overall prevalence	1(2.4%)	25(24.0%)	45(18.9%)		

Key: * =significant; chi^2 = chi-square value and p-value = point estimate value

4.3 Prevalence of Chicken Haemoparasites Based on Sex

Out of the total females examined, 53 (20.6 %) were positive of haemoparasite infection in a single and mixed infections. The recorded haemoparasite prevalence was, *Aegyptienella* spp. (7.4%), *Haemoproteus* spp. (3.9 %), *Leucocytozoon* spp. (3.1 %) and *Plasmodium* spp. (1.2 %) in single form of infection. In mixed form of infection the record was, *Aegyptienella* and *Haemoproteus* (1.6 %), *Aegyptienella* and *Plasmodium* (1.2%), *Leucocytozoon* and *Plasmodium* (0.85%), *Haemoproteus* and *Plasmodium* (0.8%), *Aegyptienella, Plasmodium and Haemoproteus* (*0*.8%). Out of the total examined male chicken, 18(14.2%) were positive of haemoparasite in a single and mixed form. The identified haemoparasites were *Aegyptienella* spp (7.9%), *Haemoproteus* spp. (0.8%), *Leucocytozoon* spp. (0.8%) in single form of infection. In mixed form of infection the observed record was,

Aegyptienella and *Plasmodium* (1.6%), *Aegyptienella* and *Haemoproteus* (1.6%), *Leucocytozoon* and *Plasmodium* (0.8%), *Aegyptienella*, *Plasmodium* and *Leucocytozoon* (0.8%). The females chickens in the study area was found 1.57 times more likely to be affected by haemoparasite infection than males chicken (OR; 1.57, 95% CI= 0.88-2.82).The association between sex was not statistically significant (x^2=2.35; P>0.05). (Table 5 and Table 11).

Table 5: Prevalence of Chicken Haemoparasites Infection Based on Sex

Haemoparasites identified	**Sex**		**Chi2**	**p-value**
	Female (n=257)	**Male (n=127)**		
Aegyptienella spp.	19(7.4%)	10(7.9%)		
Haemoproteus spp.	10(3.9%)	1(0.8%)		
Leucocytozoon spp.	8(3.1%)	1(0.8%)		
Plasmodium spp.	3(1.2%)	0(0.0%)		
Aegyptienella and Plasmodium spp.	3(1.2%)	2(1.6%)	2.346	0.126
Leucocytozoon and Plasmodium spp.	2(0.8%)	1(0.8%)		
Aegyptienella and Haemoproteus spp.	4(1.6%)	2(1.6%)		
Haemoproteus and Plasmodium spp.	2(0.8%)	0(0.0%)		
Aegyptienella, Plasmodium and Leucocytozoon	0(0.0%)	1(0.8%)		
Aegyptienella, Plasmodium and Haemoproteus	2(0.8%)	0(0.0%)		
Overall prevalence	53(20.6%)	18(14.2%)		

Key: chi^2 = chi-square value and p-value = point estimate value

3.4 Prevalence of Chicken Haemoparasites Based on the Origin of Districts

Among the three districts, the overall chicken haemoparasite infection in South Achefer was highest 35(27.3%) followed by Bahirdar Zuria 22 (17.2%) and the least infection was recorded in Mecha district 14(10.9%) in single and mixed form of infections. In the form of single haemoparasite infections the reported were *Aegyptienella* spp. (9.4 %, 8.5% and 4.6%), *Haemoproteus* spp. (2.3 %, 1.6% and 4.6%), *Leucocytozoon* spp. (4.7 %, 0.8 and 1.6%) and *Plasmodium* spp. (1.5 %, 0.0% and 0.9%) in South Achefer, Bahirdar Zuria and Mecha districts, respectively. In mixed form of infection the record was observed in S/Achefer and B/Zuria districts. The recorded haemoparasites were

Aegyptienella and *Haemoproteus* (2.3 % and 2.3%), *Aegyptienella* and *Plasmodium* (1.5% and 2.3%), *Leucocytozoon* and *Plasmodium (2*.3% and 0.0%), *Haemoproteus* and *Plasmodium* (0.9% and 0.9%), *Aegyptienella*, *Plasmodium* and *Haemoproteus* 1.5% and 0.0%), *Aegyptienella*, *Plasmodium* and *Leucocytozoon* (0.9 % and 0.0%) in S/Achefer and B/Zuria districts, correspondingly. The association among districts were statistically significant (x^2=11.65; P<0.05). Chickens in households of S/Achefer district was found 0.33 times more likely to be affected by haemoparasite infection than those chickens found in Mecha district with (OR; 0.33, 95% CI=0.11-0.17) (Table 6 and Table 11).

Table 6: Prevalence of Chicken Haemoparasites Based on the origin of Districts

Identified haemoparasites	**Districts**			**Chi^2**	**p-value**
	Mecha (n=128)	**S/Achefer (n=128)**	**B/Zuria (n=128)**		
Aegyptienella spp.	11 (8.5%)	12(9.4%)	6(4.6%)		
Haemoproteus spp.	2 (1.6%)	3(2.3%)	6(4.6%)		
Leucocytozoon spp.	1(0.8%)	6(4.7%)	2(1.6%)		
Plasmodium spp.	0(0%)	2(1.5%)	1(0.9%)		
Aegyptienella and Plasmodium spp.	0(0%)	2(1.5%)	3(2.3%)	11.65	0.003*
Leucocytozoon and Plasmodium spp.	0(0%)	3(2.3%)	0(0%)		
Aegyptienella and Haemoproteus spp.	0(0%)	3(2.3%)	3(2.3%)		
Haemoproteus and Plasmodium spp.	0(0%)	1(0.9%)	1(0.9%)		
Aegyptienella, Plasmodium and Leucocytozoon	0(0%)	1(0.9%)	0(0%)		
Aegyptienella, Plasmodium and Haemoproteus	0(0%)	2(1.5%)	0(0%)		
Overall prevalence	14(10.9%)	35 (27.3%)	22(17.2%)		

KEY: * =significant; Chi^2= chi-square value; P-value =point estimate value, n= number of sampled

4.5 Prevalence of Chicken Haemoparasites Based on the Origin of PA's

Among the six PA's, the overall prevalence of chicken haemoparasite infection in Kare was the highest 20(31.3%), followed by Dibikan 15(23.4%) next was Sebatamit 12(18.8%), then Felegebirhan, Yibab 10 (15.6%) 8(12.5%), and the least infection was Wotet ber 6 (9.4%). The haemoparasite infections were identified in the form of single infection in all six PA's and the mixed type of haemoparasite infections were recorded in only the four PA's of Dibikan, Kare, Yibab and Sebatamit. The difference in overall prevalence of haemoparasite infection among PA's were

statistically significant (chi^2= 13.36; P<0.005). Chickens in households of Kare PA's was found 0.23 times more likely to be affected by haemoparasite infection than chicken found in Wotetber PA's with (OR; 0.23, 95% CI: 0.08-0.63). (Table 7 and Table11).

Table 7: Prevalence of Chicken Haemoparasites Based on the Origin of PA's

Identified Haemoparasites	**Peasant associations**						**Chi²**	**p-value**
	Dibikan (n=64)	**F/birhan (n=64)**	**Kare (n=64)**	**Sebatam (n=64)**	**W/be (n=64)**	**Yibab (n=64)**		
Aegyptienella spp.	8(12.4%)	6(9.3%)	4(6.3%)	3(4.7%)	5(7.8%)	3(4.7%)		
Haemoproteus spp.	1(1.6%)	1(1.6%)	2(3.0%)	3(4.7%)	1(1.6%)	3(4.7%)		
Leucocytozoon spp.	2(3.0%)	1(1.6%)	4(6.3%)	0(0.0%)	0(0.0%)	2(3.1%)		
Plasmodium spp.	1(1.6%)	0(0.0%)	1(1.6%)	1(1.6%)	0(0.0%)	0(0.0%)		
Aegyptienella and Plasmodium	1(1.6%)	0(0.0%)	1(1.6%)	3(4.6%)	0(0.0%)	0(0.0%)	13.36	0.02
Leucocytozoon and Plasmodium	1(1.6%)	0(0.0%)	2(3.0%)	0(0.0%)	0(0.0%)	0(0.0%)		
Aegyptienella and Haemoproteus	0(0.0%)	0(0.0%)	3(4.7%)	1(1.6%)	0(0.0%)	2(3.1%)		
Haemoproteus and Plasmodium	0(0.0%)	0(0.0%)	1(1.6%)	1(1.6%)	0(0.0%)	0(0.0%)		
Aegyptienella, Plasmodium, Leucocytozoon	0(0.0%)	0(0.0%)	1(1.6%)	0(0.0%)	0(0.0%)	0(0.0%)		
Aegyptienella, Plasmodium, Haemoproteus	1(1.6%)	0(0.0%)	1(1.6%)	0(0.0%)	0(0.0%)	0(0.0%)		
Overall prevalence	15(23.4%)	8(12.5%)	20(31.3%)	12(18.8%)	6(9.4%)	10(15.6%)		

KEY: * =significant; Chi^2= chi-square value; P-value =point estimate value.

4.6 Prevalence of Chicken Haemoparsites Based on Different Agroecological Zone

In the present study, overall prevalence of the haemoparasite infection was highest in the midland areas of 57(22.3%) and the least infection was found in highland 14 (10.9%). Chickens in households of midland area was found 0.42 times more likely to be affected by haemoparasite infection than the chickens found in the highland areas with (OR; 0.42, CI=0.229-0.804). The difference in overall prevalence of haemoparasite infection between midland and highland was statistically significant (x^2 =11.65; P< 0.05) (Table 8 and Table11).

Table 8: Prevalence of Chicken Haemoparasites Based on Agroecological Zones

Identified Haemoparasites	**Agroecology**		**Chi2**	**P-value**
	Highland (n=128)	**Midland (n=256)**		
Aegyptienella spp.	11 (8.5%)	18(7.0%)		
Haemoproteus spp.	2 (1.6%)	9(3.5%)		
Leucocytozoon spp.	1(0.8%)	8(3.1%)		
Plasmodium spp.	0(0%)	3(1.2%)		
Aegyptienella and Plasmodium spp.	0(0%)	5(2.0%)	11.65	0.003*
Leucocytozoon and Plasmodium spp.	0(0%)	3(1.2%)		
Aegyptienella and Haemoproteus spp.	0(0%)	6(2.3%)		
Haemoproteus and Plasmodium spp.	0(0%)	2(0.8%)		
Aegyptienella, *Plasmodium* and *Leucocytozoon*	0(0%)	1(0.4%)		
Aegyptienella, *Plasmodium* and *Haemoproteus*	0(0%)	2(0.8%)		
Overall prevalence	14 (10.9 %)	57 (22.3%)		

KEY: * =significant; Chi^2= chi-square value; P-value =point estimate value, n= number of sampled

4.7 Prevalence of Chicken Haemoparasites Based on the Type of Breeds

Throughout the study period, overall prevalence of the hamoparasites infection was highest in local indigenous breeds 63(18.6%) than cross breeds 8(17.4%). The local indigenous chickens in the study area was found 1.09 times more likely to be affected by haemoparasite infection than chickens of cross breed with (OR; 1.09, 95% CI= 0.45-2.45) .The difference in overall prevalence of haemoparasite infection between indigenous and cross breed was not statistically significant (X^2= 2.50; p>0.05) (Table 9 and Table11).

Table 9: Prevalence of Chicken Haemoparasites Based on the Type of Breeds

Identified Haemoparasites	**Breed**		**Chi^2**	**p-value**
	Indigenous (n=338)	**Cross (n=46)**		
Aegyptienella spp.	26(7.7%)	3(6.5%)		
Haemoproteus spp.	9(2.6%)	2(4.3%)		
Leucocytozoon spp.	8(2.3%)	1(2.2%0		
Plasmodium spp.	3(0.9%)	0(0.0%)		
Aegyptienella and Plasmodium spp.	4(1.2%)	1(2.2%)	2.50	0.981
Leucocytozoon and Plasmodium spp.	3(0.9%)	0(0.0%)		
Aegyptienella and Haemoproteus spp.	5(1.5%)	1(2.2%)		
Haemoproteus and Plasmodium spp.	2(0.6%)	0(0.0%)		
Aegyptienella, Plasmodium and Leucocytozoon	1(0.3%)	0(0.0%)		
Aegyptienella, Plasmodium and Haemoproteus	2(0.6%)	0(0.0%)		
Overall prevalence	63(18.6%)	8(17.4%)		

KEY: Chi^2= chi-square value; P-value =point estimate value, n= number of sampled

4.8 Prevalence of Haemoparasite Infection Based on Different Management Systems

In this study, overall prevalence of the hamoparasites infection was highest in extensive management system 70 (21.2%) and the least infection was observed chickens were in under semi extensive management system 1(1.9 %). Chickens in under extensive management system was found 14.3 times more likely to be affected by haemoparasite infection than chickens in under semiextensives of the study area with (OR; 14.3, 95% CI: 1.40-105.0) (Table 11).The difference in overall prevalence of haemoparasite infection between extensive and semi extensive management system was statistically significant (X^2= 3.97; p< 0.05) (Table 10 and Table11).

Table 10: Prevalence of Haemoparasites in Different Management System

Identified parasites	**Management system**		**Chi2**	**p-value**
	Extensive (n=330)	**S/ extensive (n=54)**		
Aegyptienella spp.	28(8.5%)	1(1.9%)		
Haemoproteus spp.	11(3.3%)	0(0.0%)		
Leucocytozoon spp.	9(2.7%)	0(0.0%)		
Plasmodium spp.	3(0.9%)	0(0.0%)		
Aegyptienella and *Plasmodium* spp.	5(1.55)	0(0.0%)		
Leucocytozoon and *Plasmodium* spp.	3(0.9%)	0(0.0%)	3.97	0.000*
Aegyptienella and *Haemoproteus* spp.	6(1.8%)	0(0.0%)		
Haemoproteus and *Plasmodium* spp.	2(0.6%)	0(0.0%)		
Aegyptienella, Plasmodium and Leucocytozoon	1(0.3%)	0(0.0%)		
Aegyptienella, Plasmodium and Haemoproteus	2(0.6%)	0(0.0%)		
Overall prevalence	70(21.2%)	1(1.9%)		

KEY: * =significant; Chi^2= chi-square value; P-value =point estimate value, n= number of sampled

Table 11: Haemoparasite Infection with Association of Risk Factors by Univariable Logistic Regression

Risk factors	No of sampled	Positive	Prevalence %	OR	95% CI		P-value
					Lower	Upper	
Age							
Chicks	42	1	2.4	RF	-	-	-
Adult	238	45	18.9	0.93	1.269	72.8	0.028*
Grower	104	25	24.0	0.66	0.366	1.19	0.16
Sex							
Male	127	18	14.2	RF	-	-	-
Female	257	63	18.6	1.57	0.878	2.818	0.128
Districts							
Mecha	128	14	10.9	RF	-	-	-
B/Zuria	128	22	17.2	0.591	0.2174	0.288	0.153
S/Achefer	128	35	27.3	0.326	0.113	0.166	0.001*
Agroecology							
Highland	128	14	10.9	RF	-	-	-
Midland	256	57	22.3	0.429	0.229	0.804	0.008*
PA's							
WB	64	6	9.4	RF	-	-	-
FB	64	8	12.5	0.778	0.247	2.45	0.667
YIB	64	10	15.6	0.633	0.202	1.80	0.364
SEB	64	12	18.8	0.481	0.17	1.40	0.177
DIB	64	15	23.4	0.405	0.14	1.50	0.089
Kare	64	20	31.3	0.230	0.08	0.63	0.004*
Management							
S/ extensive	54	1	1.9	RF	-	-	-
Extensive	330	70	21.2	14.3	1.40	105.0	0.000*
Breed							
Cross	46	8	12.5	RF	-	-	-
Indigenous	338	63	20.6	1.09	0.484	2.45	0.838

KEY: CI=confidence interval, OR= odds ratio, P-value= point estimate value, RF=Reference

DIB= Dibkan, FB= Felege Birhan, SEB=Sebatamit, WB= Wotet Ber, YIB= Yibab

4.9 Hematological Findings of Infected and Non-infected Chickens

A total of 142 hematology analysis from non-infected (n=71) and from chickens infected with haemoparasites (n=71) were done. The mean haematological values of RBC, PCV, Hb, MCV, MCH, MCHC, and WBC were analyzed to compare the haematological changes of the infected with non-infected chickens of both sex and all age groups. The haematologic findings were showed that, the mean RBC, PCV, Hb, MCV, and MCH value of the infected chicken was lower than the non-infected chickens, but the mean MCHC values of infected chicken and non-infected chickens were similar. The mean total white blood count of chickens infected with haemoparasite was higher than the non-infected chicken. The mean RBC and Hb were statistically significant ($p<0.05$). Nevertheless, PCV, MCV, WBC, MCH and MCHC recorded mean value was not statistically significant ($p> 0.05$) (Table 12).

4.9.1 Comparison of the Total RBC count of Infected and Non-infected Chicken

The average mean of total RBC count of the infected and non-infected chicken were 2.9 at a standard error of (2.9±0.055) with 2.7 lower and 3.0 upper CI, it was statistically significant ($p<0.05$).The mean of total RBC count of the infected chicken were 2.9 at a standard error (2.9±0.055) with 2.7 lower and 3.0 upper limit, the mean result was lower than from the non-infected chicken but it was within the normal range. For the meantime, the mean total RBC count of the non-infected chicken were 3.0, at a standard error of (3.0±0.55) with 2.7 lower limit and 3.1 was the upper limit. The mean total RBC count of the non-infected were higher than the infected but within the normal range (Table 12).

4.9.2 Comparison of the PCV measure of Infected and Non-infected Chicken

The average mean of PCV of the infected and non-infected chicken were 34.7 at a standard error of (34.7±0.608) with 32.2 lower and 34.6 upper CI, it was statistically not significant ($p>0.05$).The mean of PCV measure of the infected chicken was 33.4 at a standard error (33.4±0.608) with 32.2 lower and 34.6 upper limit, the mean PCV result was lower than from the non-infected chicken but it was within the normal range. For the meantime, the mean PCV measure of the non-infected chicken was 35.9, at a standard error of (35.9±0.885) with 34.1 lower limit and 37.7 was the upper limit. The mean PCV measure of the infected chickens were higher than the non infected (Table 12).

4.9.3 Comparison of the Hb Level of Infected and Non-infected Chicken

The average mean Hb level of the infected and non-infected chickens were 13.6 dl/g at a standard error of (13.6±0.319) with 12.4 lower and 13.7 upper CI, it was statistically significant ($p<0.05$). The mean Hb level of the infected chicken was 13.1 at a standard error (13.1±0.319) with 12.4 lower and 13.7 upper confidence level. The mean Hb level of chickens infected with haemoparasites were lower than from the non-infected chicken but it is within the normal range. For the meantime, the mean Hb level of the non-infected chickens were 14.1, at a standard error of (14.1±0.367) with 13.4 lower confidence level and 14.9 was the upper limit. (Table 12).

4.9.4 Comparison of the MCV of Infected and Non-infected Chicken

The average mean MCV of the infected and non-infected chickens were 118.3 fl at a standard error of (118.2±0.966) with 116.4 lower and 120.2 upper CI, it was statistically not significant ($p>0.05$).The mean MCV of the infected chickens were 118.2 at a standard error (118.2±1.462) with 115.3 lower and 121.1 upper confidence level, the mean MCV of the infected chicken was lower than from the non-infected chicken but it was within the normal range. For the meantime, the mean red blood cell MCV of the non-infected chicken were 118.3, at a standard error of (118.3±1.274) with 115.8 lower confidence level and 120.9 was the upper limit. (Table 12).

4.9.5 Comparison of MCH of Infected and Non-infected Chicken

The average mean MCH of the infected and non-infected chickens were 46.1% at a standard error of (46.1±0.474) with 45.2 lower and 47.1 upper CI, it was statistically not significant ($p>0.05$).The mean MCH of the infected chickens were 45.7 at a standard error (45.7±0.626) with 44.5 lower and 47.0 upper confidence level, The mean MCH of infected chicken was lower than from the non-infected chicken nevertheless it was within the normal range. For the meantime, the mean of the non-infected chicken was 46.5 at a standard error of (46.5±0.713) with 45.1 lower confidence level and 48.0 was the upper limit (Table 12).

4.9.6 Comparison of MCHC of Infected and Non-infected Chicken

The average mean MCHC of the infected and non-infected chickens were 39.0 at a standard error of (39.0±0.420) with 37.9 lower and 40.1 upper CI, it was statistically not significant ($p>0.05$).The mean MCHC of the infected chickens were 39.0 at a standard error (39.0±0.562) with 37.9 lower and 47.0

upper confidence level. The mean MCHC of both infected and non- infected were alike. But mean MCHC value was within the normal range. For the meantime, the mean MCHC of the non-infected chicken was 39.0, at a standard error of (39.0±0.628) with 37.8 lower limit and 40.3 was the upper limit (Table 12).

4.9.7 Comparison of the Total WBC Count of Infected and Non-infected Chicken

The average mean of total WBC count of the infected and non-infected chickens were 6.9 at a standard error of (6.9±0.140) with 6.6 lower and 7.1 upper CI, it was statistically not significant (p>0.05). (Table 12).

Table 12: Hematology Results of the Infected and the Non-infected Chicken

Parameters		**Observations**	**Mean**	**M ±SE**	**95% CI**		**P-value**
					Lower	**Upper**	
RBC $\times 10^{12}$	infected	71	2.9	2.9±0.055	2.7	3.0	
	non infected	71	3.0	3.0±0.055	2.9	3.1	0.0317*
	combined	142	2.9	2.9±0.039	2.9	3.0	
PCV/%	infected	71	33.4	33.4±0.608	32.2	34.6	
	non infected	71	35.9	35.9±0.885	34.1	37.7	0.223
	combined	142	34.7	34.7±0.545	33.6	35.7	
Hb g/dl	infected	71	13.1	13.1±0.319	12.4	13.7	
	non infected	71	14.1	14.1±0.367	13.4	14.9	0.034*
	combined	142	13.6	13.6±0.246	13.1	14.1	
MCV fl	infected	71	118.2	118.2±1.462	115.3	121.1	
	non infected	71	118.3	118.3±1.274	115.8	120.9	0.9503
	combined	142	118.3	118.3±0.966	116.4	120.2	
MCH pg	infected	71	45.7	45.7±0.626	44.5	47.0	
	non infected	71	46.5	46.5±0.713	45.1	48.0	0.3872
	combine	142	46.1	46.1±0.474	45.2	47.1	
MCHC dl	infected	71	39.0	39.0±0.562	37.9	40.1	0.9947
	non infected	71	39.0	39.0±0.628	37.8	40.3	
	combined	142	39.0	39.0±0.420	38.2	39.8	
WBC$\times 10^{3}$	infected	71	6.8	6.8±0.210	6.4	7.2	
	non infected	71	6.9	6.9±0.188	6.5	7.3	0.7431
	combined	142	6.9	6.9±0.140	6.6	7.1	

KEY: CI= Confidence Interval; P =point estimate value, SE= Standard Error

4.10 Hematological Findings of Infected Chicken According to Level of Infection

The average mean of total RBC count of the chickens infected with single haemoparasite were 2.8 at a standard error of (2.8±0.101) with 2.7 lower and 3.0 upper CI, the mean result of RBC count was higher than chickens infected with mixed form parasites. The association between them was not statistically significant (p>0.05).The mean PCV value of red blood cells of the chickens infected with single haemoparasites were 33.5% at a standard error (33.5±0.959) with 32.0 lower and 35.0 upper limit, the mean result was higher than from the chickens infected with more than one haemoparasites. Nevertheless, mean PCV value was within the normal range statistically not significant (p>0.05) (Table 13). The hemoglobin level of chickens infected with single haemoparasite were lower than the Hb level of infected chickens with more than one haemoparasites.

The recorded results of the mean Hb level was 13.0 g/dl at a SE (13.0±0.383) with 12.2 lower and 13.7 higher CI; 13.5 g/dl at a SE (13.5±2.557)With 12.3 lower and 14.6 higher CI, in chickens infected with one and more than one haemoparasites, respectively. The Hb level of both infected and non-infected chickens were within the normal range. It was not statistically significant (P>0.05) (Table 13).

The mean MCV value of red blood cells of the chickens infected with single haemoparasites were 118.7% at a standard error (118.7±1.753) with 115.2 lower and 122.2 upper limit, the mean result was higher than from the chickens infected with more than one haemoparasites. But it was beyond the normal range and statistically not significant (p>0.05) (Table 12).The MCH value of chickens infected with single haemoparasite were lower than the MCH value of infected chickens with more than one haemoparasites. The observed results of the MCH was 45.3% at a SE (45.3±0.783) with 43.9 lower and 46.8 higher CI; 46.9% at a SE (46.9±1.148) With 44.5 lower and 49.3 higher CI in chickens infected with one and more than one haemoparasites, respectively. The MCH value of both infected and non infected chickens were within the normal range. It was statistically not significant (P>0.05) (Table 13).

The MCHC value of chickens infected with single haemoparasite was lower than the MCHC value of infected chickens with more than one haemoparasites. The observed results of the MCHC was 38.6% at a SE (38.6±0.629) with 37.3lower and 39.8 higher CI; 40.4% at a SE (40.4±1.110) With 37.9 lower and 43.0 higher CI in chickens infected with single and more than one haemoparasites, respectively.

The MCH value of both infected and non infected chickens were within the normal range. It is statistically not significant (P>0.05).

For the meantime, the mean total WBC count of chickens infected with single haemoparasites were 6.8, at a standard error of (6.8±0.247) with 6.3 lower limit and 7.3 was the upper limit. The mean total WBC count of the chickens infected with single haemoparasite were analogous with mixed haemoparasite infected chickens. On the other hand, the mean TWBC count was within the normal range and statistically was not significant (p>0.05) (Table 13).

Table 13: Hematology Results of Infected Chicken in According to Level of Infection

Parameters		**Observations**	**Mean**	**M± SE**	**95 % CI**		**P-value**
					Lower	**Upper**	
RBC × 10^{12}	single	52	2.8	2.8±0.101	2.7	3.0	
	mixed	19	2..9	2.9±0.060	2.7	3.0	0.162
	total	71	2.9	2.9±0.055	2.7	3.0	
PCV%	single	52	33.5	33.5±0.959	32.0	35.0	
	mixed	19	33.2	33.2±0.745	31.2	35.2	0.2753
	total	71	33.4	33.4±0.608	32.2	34.6	
Hb g/dl	single	52	13.0	13.0±0.383	12.2	13.7	
	mixed	19	13.5	13.5±0.558	12.3	14.6	0.1883
	total	71	13.1	13.1±0.319	12.4	13.7	
MCV fl	single	52	118.7	118.7±1.753	115.2	122.2	
	mixed	19	116.7	116.7±2.557	11.3	122.1	0.2468
	total	71	118.2	118.2±1.462	115.3	121.1	
MCH pg	single	52	45.3	45.3±0.738	43.9	46.8	
	mixed	19	46.9	46.9±1.148	44.5	49.3	0.6949
	total	71	45.7	45.7±0.626	44.5	47.0	
MCHC dl	single	52	38.6	38.6±0.629	37.3	39.8	
	mixed	19	40.4	40.4±1.199	37.9	43.0	0.3188
	total	71	39.0	39.0±1.307	37.9	40.1	
WBC×10^{3}	single	52	6.8	6.8±0.247	6.3	7.3	
	mixed	19	6.8	6.8±0.407	5.9	7.7	0.0925
	total	71	6.8	6.8±0.210	6.4	7.2	

KEY: M=mean; P-value= point estimate value, SE= standard error

4.11 Questionnaire Survey

Individual conversations with farmers of both sex and having dissimilar educational status were conducted with assistance from the livestock and fishery agency staffs of Mecha, S/Achefer and B/Zuria districts between Decembers to February 2017. Consistent questionnaires were engaged to 108 randomly selected households from PA's of Mecha (n=36), S/Achefer (n=36) and B/Zuria (n=36). The sex of the respondent farmers were, males (41.7%, 50% and 36.1%) and females were (58.3%, 50%, 63.9%) as well, educational status of the respondent farmers were illiterates (36.1%, 58.3%, 44.5) and write read (63.9%, 41.7%, 55.5%) in Mecha, S/Achefer and B/Zuria districts, respectively. The assessment was showed that the majority of the respondent were females (57.5%) (Table 14a). This point out that most of the time the women are responsible for the rising of chicken, even though, men are responsible for growing of crops. The questionnaires were semi structured with both closed and open ended questions that were designed to acquire information on the village chicken production systems with emphasis on backyard growing system by the farmers, flock size of the chickens and access of availability of diverse chicken breeds, roles of village chicken growing, chicken nutrition; housing systems of the chicken; health management as well as commonly occurring chicken diseases with observed clinical signs and mode practices of drug administration while the chicken became sick (Table 14).

4.11.1 Village Chicken Flock Size and Composition

A total of 452, 265 and 222 village chickens were reported by the farmers in Mecha, S/Achefer and B/Zuria districts respectively. Flock sizes varied between growers in Mecha, S/Achefer and B/Zuria districts and about 75% of the farmers from all districts had between 0 - 10 chickens. Twenty percent of the growers owned 10-20 chickens and the remaining 5% owned 40 chickens in all districts. Different types of breeds of chicken was reported by the respondents of which were, indigenous breeds (71.7%); cross breeds (15.1%) and exotics (13.2%) in all districts. Distinctly, in Mecha (82.3%, 4.4% and 13.3%); S/Achefer (72.1%, 17.7% and 10.2%) and B/Zuria (60.8%, 23.3% and 15.9%) were indigenous, cross and exotics, respectively (Table 14).

4.11.2 Role of Rising Chicken in Study Areas

Results of the questionnaire survey was revealed that rising of chicken in the study area by the farmers were mainly for income generation by selling the chicken and their eggs were 70(64.8%), for the

purpose of house hold consumption as a source of food 27(25%) and for both income generation and house hold consumption was 11(10.2%) (Table 14).

4.11.3 Commonly Observed Chicken Diseases, Clinical Signs and their Measures

Around 67(62%) of the respondents were reported that Newcastle disease was the most important constraint that cause chicken mortality in all districts followed by unknown cases 23(21.3%) and the least respondents were reported 18(16.7%) as parasitic cases. As of the total respondents of the districts in Mecha, South Achefer and Bahirdar Zuria, Newcastle disease 22(61.1%); 24(66.7%) and 21(58.3%); parasites 5(13.9%); 7(19.4%) and 4(11.1%) as well as unknown cases 9(25%); 5(13.9%) and 11(30.6%) were reported, respectively. The most predominant chicken disease clinical signs observed by farmers in reducing order of importance in both districts the leading was, diarrhoea (63.9%, 72.2% and 75%) tailed by ruffielled feather (22.2%, 27.8%, 25%) and the least record was torticollis (13.9%, 0% and 0%). In addition to this, the respondents were reported that, taken measures of control when the chicken becomes affected by the disease was commercially available oxy tetracycline powders 84(77.8%) and some of them was used traditional medicines 24(22.2%) as preventives and curative purposes (Table 14).

4.11.4 Management System of Chickens

The chickens were allowed to scratch freely in the open areas near the home and surroundings. From the result of questionnaire assessment in the study area that, majority of the chickens are managed under a traditional or extensive chicken management system 83(76.9%) and some of them was fed different varieties of cereals 25(23.1%) by mixing them. Almost all the farmers in the study area provided supplementary feeding to their chickens of different groups of age were fed together. However, the type and amount of feed depend on the crops grown in the area. The majority of the farmers who accomplished supplementary feeding systems used maize, dagusa, barely and house hold waste products to feed their chicken. The questionnaire survey was indicated that almost all farmers provided mixed shelter for their chickens 71(65.7%), either in part of the kitchen or in the main house or in separate shelter 37(34.3%) purpose made for chickens. These shelters were made of locally available materials such as wood, mud and corrugated iron sheet. This is an indication that the owners are awake of the importance of housing (Figure 17). The respondent was reported that the management system of indigenous chicken is not improved. Based on the respondent information, mixed type of

housing system and free scavenging was dominated over separated housing system, preparation of the chicken nutrition as well as using of feed troughs in the study area. About 78(72.2%) of the farmers were provided feed and water on the ground as scavenging freely and 30(27.8%) was implemented the feeding system in troughs made from wood, clay materials (bowl), piece of plastics and commercially available materials (Table 14).

Table 14: Summary of Questionnaire Survey in the Study Area

Parameters	**Study areas**						**Mean**
	Mecha (n=36)		**B/Zuria (n=36)**		**S/Achefer (n=36)**		
	Frequency	**%**	**Frequency**	**%**	**Frequency**	**%**	
Purpose of rising chicken							
Cash income	22	61.0*	23	63.9*	25	69.4*	23.3(64.8%)*
Consumption	8	22.0	8	22.0	11	30.6	9 (25 %)
Both	6	17.0	5	14.1	0	0.0	3.7(10.2%)
Breed of chicken							
Local	372	82.3*	141	60.8*	191	72.1*	234.7(71.7%)*
Cross	20	4.4	54	23.3	47	17.7	40.3 (15.1%)
Exotics	60	13.3	37	15.9	27	10.2	41.3 (13.2%)
Number of chicken owned							
Local							
Male	44	11.8	67	45.5	35	18.3	48.7(25.3%)
Female	228	88.2*	74	52.5	156	81.7	152.7(74.7%)*
Total	372		141		191		
Cross							
Male	5	25.0	17	31.5	11	23.4	11(26.6%)
Female	15	75.0*	37	68.5	36	76.6	29.3(73.4%)*
Total	20		54		47		
Exotic							
Male	3	5.0	0	0	0	0	1(1.7%)
Female	57	95.0*	37	100*	27	100*	40.3 (98.3%)*
Total	60		37		27		
Feed preparation							
Traditionally	26	72.2*	27	75.0*	30	83.3*	27.7(76.9%)*
Mixing of cereal	10	27.8	9	25.0	6	16.7	8.3(23.1%)
Feeding systems							
On ground	26	72.2*	22	61.1*	30	83.3*	26 (72.2%)*
Troughs	10	27.7	14	38.9	6	16.7	10(27.8%)
Commonly using drug							
Commercial	30	83.3*	28	77.8	26	72.2*	28(77.8%*
Traditionally	6	16.7	8	22.2	10	27.8	8(22.2%)
Common c/ signs							
Diarrhea	23	63.9*	27	75.0*	26	72.2*	25.3(70.3%)*
R/feather	8	22.2	9	25.0	10	27.8	9(25%)
Torticollis	5	13.9	0	0.0	0	0.0	1.7(4.7%)

KEY: *= shows higher percentage, n=number of sample

5. DISCUSSION

At the study period four chicken haemoparasites viz. *Aegyptianella*, *Haemoprotues*, *Leucocytozoon* and *Plasmodium* species were found to infect indigenous chickens in the form of single infections of the study area. There was also mixed infections among *Aegyptienella*, *Haemoproteus*, *Leucocytozoon* and *Plasmodium* species. This means that suitable arthropod vectors (*Argas persicus*, Hippoboscide, Mosquitoes, *Simulidae*, and Cullicoides) were common throughout the study areas. The result of this study revealed the prevalence of chicken haemoparasites in village chickens in three selected districts of West Gojjam Zone, Amhara regional state which have different agroecological zones. Out of 384 blood samples collected and microscopically identified for the presence of chicken haemoparsites, 71 chickens were infected with an overall prevalence of 18.5%.

The result of this study revealed an overall prevalence of 18.5%.haemoparasite infection of chickens in the study area. Likewise, the current study was in agreed with the findings of Opara *et al*. (2016) with prevalence of 12% in Nigeria. However, the present finding in the prevalence of chicken haemoparasite is much lower than the findings of Sabuni *et al*. (2011), Shadan, (2013) and Hasson, (2015) with a prevalence of 79.2%, 78.2% and 76% in Kenya, Qaradagh district of Iraq, respectively. As well, the result reported by Emebet (2017), Poulsen *et al*. (2000) and Permin *et al*. (2002) in Ethiopia, Ghana and Zimbabwe, who reported 43.4%, 35% and 32%, respectively was higher than the current study result. These variations among the present and previous studies may be due to the differences in geographic locations, breeds of chicken, management factors, season of availability of insect vectors and method of study, sample size difference.

In the present study, the prevalence of *Aegyptenella* spp. was 28.6% in chickens. This result is in line with the findings of Njunga (2003) as 25% in Malawi. However, higher rate of infection was recorded by Sehgal *et al*. (2006) in Uganda and Alex (2009) in Kenya with a prevalence of 31%, and 52.1% respectively. A lower result prevalence of *Aegyptenella* spp than the present study was recorded by Permin *et al*. (2002) in Zimbabwe and Emebet (2017) in Ethiopia with a prevalence of 13.8% and 10.4%, respectively. These deviation may be due to sample size difference, seasonal difference of the study accessibility of arthropod vector fowl soft tick, poor management system and lack of owner's awareness to get treatment of the infested chickens with acaricides.

In the current study, the prevalence of *Leucocytozoon* spp. was 7.5% in the study chickens of the study areas. The existing study is in line with the reports of Opara *et al.* (2014) in Nigeria and Emebet (2017) with a prevalence of 8.9% and 9.6%. However, this study result is lower than the findings of Sehgal *et al.* (2006) in Uganda, Alex (2009) in Kenya and Abdul *et al.* 2014) in Bangladesh with a prevalence of 31%, 52.1% and 34.6%), respectively. Meanwhile, the findings of Permin *et al*. (2002) in Zimbabwe were lower (4.3%) than the current study. These variations might be due to the geographic location, management systems, and methodology of the study.

This study revealed a prevalence of 5.7% in *Plasmodium* spp. which is comparable with the study result of Poulsen *et al*. (2002), with a prevalence of 2.5% in Ghana. Unlikely, Permin *et al.* (2002) in Zimbabwe Njunga (2003) in Malawi, Alex (2009) in Kenya and Emebet (2017) in Ethiopia, with a prevalence of, 14.9%, 15% and 53.7% and 18.5% respectively. These variations might be due to the geographic location, management systems, methodology of the study and accessibility of insect vectors in the study areas.

In the present study, the prevalence of *Haemoproteus* spp. was 12.3% in chickens. This result agreed by Hassan (2015), with a prevalence of 13.2% at the province of Diyalla in Iraq. Higher rate of infection was recorded by Lawal *et al*. (2016), 50.9%, in Nigeria and Ikhlas (2017), 33.8%, in Iraq. In Kenya, a lower prevalence (3.5%) than the present study was found by Sabuni *et al*. (2011). The differences may be due to the fact that the finding in one area in their native chicken while, the others may not be. It may be attributed to the management practices, geographic locations, and season of the year that the researcher was carried out. Our research was done during non-rainy season, as this is the period that the vector of most chicken haemoparasite not thrive well. The tropical rain forest vegetation favors the breeding and multiplication of haemoparasites and their vectors (Opara *et al*., 2009).

In the present study, the prevalence of chicken haemoparasites in different age groups were higher in growers (24%) followed by adult chicken (18.9%) and the least infection was recorded in chicks with (2.4%) prevalence. This study is in line with the previous findings in Tanzania with prevalence of (16.3%) adults, (15%) growers and (5%) chicks by Swai *et al*. (2010). Nevertheless the current study is more likely lower than the previous findings of Sabuni *et al*. (2011) and Emebet (2017) with prevalence of adults (81.3% and 45.2%), growers (83.3% and 40.5%) and chicks (72.9% and 42.3%)

in Kenya and Ethiopia, respectively. The causes of higher haemoparasite infection in adult and grower chicken and chicks are less exposed to haemoparasite infection. It might be, the chicks are protected by their mother from the insect vectors with embracing (covering the chicks in their wings) the chicks, less mobile and short contact time but adults and grower chickens are highly mobile and have long contact time between vectors while scavenging.

From the present study, it was detected that the prevalence of chicken haemoparasite was higher in females (20.6%) than males (14.2%). This finding is more or less similar to the finding of Swai *et al*. (2010) and Lawal *et al*. (2016) in Tanzania and Nigeria with prevalence of males (15.9% and 20.5%) and females (15.1% and 11.5%), respectively. On the contrary, higher prevalence was reported by Sabuni *et al*. (2011), Opara *et al.* (2016) and Emebet (2017) in Kenya, Nigeria and Ethiopia with prevalence of males (83.3%, 33.3% and 45.1%) and females (75%, 66.7% and 43.1%), respectively. The exact cause of higher blood parasite infection in females cannot be explained but, in general higher level of prolactin and progesterone hormone suppress the immune status of the individual chicken and make the female chicken more susceptible than males to any parasite infection (Lloyd, 1983).

In this study, the level of haemoparasite infection was recorded as single, mixed and triple form of haemoparasite infection, the reported prevalence was 73.2%, 22.6% and 2.8%, respectively. The current finding was agreed with the form of infections, with the finding of Hasson, (2015), but different in prevalence percentage with as single (15.8%), mixed (47.4%) and triple form of infection (36.8%) in Iraq. On the other hand, two form of haemoparasitic infections are reported in lower and higher rate of infection by Sabuni *et al*. (2009), Gimba *et al*. (2014), Emebet (2017) and Lawal *et al*. (2016) as single form of infection (62.3%, 85.7%, 88% and 82.3%) and mixed form of infection was (37.7%, 14.3%, 12%and 17.6%) in Kenya, Malaysia, Ethiopia and Tanzania, respectively. Several endogenous and exogenous factors have an accumulative influence on the parasitisation of chicken by these parasites, such as host's hormones and humeral compounds, age and nutritional state, behavior and habits, as well as the season of the year and ecological variation and physical features of the areas (Hasson, 2015).

During this study, the haemoparasite infection percentage record was higher in the midland areas (44.5%) than the highlands (10.9 %). Based on the present record the recorded prevalence was,

Aegyptienella spp. (14% , 8.5%), *Haemoproteus* spp.*(*6.9%,1.6%), *Leucocytozoon* spp.(6.3%, 0.8%), *plasmodium* spp. (2.4%, 0.0%), *Aegyptienella* and *Plasmodium* (3.8%, 0.0%), *Leucocytozoon* and *Plasmodium* (2.3%, 0.0%), *Aegyptienella* and *Haemoproteus* (4.6%, 0.0%), *Haemoproteus* and *Plasmodium* (1.8%, 0.0%), *Aegyptienella, Plasmodium and Leucocytozoon* (0.9%, 0.0%) and *Aegyptienella, Plasmodium and Haemoproteus* (1.5%, 0.0%) in midland and highland study areas, respectively. The current result is slightly similar in haemoparasite occurrence but lower than the finding of Sabuni *et al*. (2011), with prevalence of 81.9% (*Plasmodium* spp. 61.1% and *Leucocytozoon* spp. 51.4%) and 76.4% (*Plasmodium* spp. 76.4% and *Leucocytozoon* spp. 52.8%) in highland areas and in midlands, respectively. This variation for the occurrence of haemoparasite infection in different agro climate might be, the availability of insect vectors need ambient temperature, and humidity and moisture to bred and multiply.

From the present-day study, it was observed that the prevalence of chicken haemoparasite was higher in chickens under extensive management (21.2%) than semiextensives (1.9%). This identification is dissimilar with the finding of Permin *et al*. (2002) in Zimbabwe, Poulsen *et al.* (2000) in Ghana and Emebet (2017) in Ethiopia. Most of the previous studies were focused only in which chickens are under extensive management systems. The distinctions of the manifestation of the haemoparasite infections might be chickens which are under semi-extensive management systems may have better immune status than the freely scavenging chickens. On the other hand chickens in under extensive management system get bitten by hematophagous flies with in the environment while scavenging for feed.

From the existing study, it was observed that the prevalence of chicken haemoparasite was higher in local breeds (20.6%) than cross breed chickens (12.5%) in prevalence. Most of the previously reported studies were concentrated only indigenous chickens and there was no reported studies on cross breed chickens. But many of the reported studies were only in local pure breeds of chicken. The local pure chicken breeds were infected more with haemoparasites than cross breeds. This might be recognized to the fact that the cross breed chickens are raised semi extensively in confinement with fly screens, thus limiting their contact with the vectors of these haemoparasites. Besides this the local pure breed chickens get bitten by arthropod vectors with in the environment when scratching to obtain feed.

Hematological parameters in chickens had been shown to be influenced by various factors such as age, sex, season and nutrition (Pavlak *et al*., 2005). In general haematological parameters were affected by diurnal fluctuations or changes in daily physical and metabolic activities (Sanni *et al*., 2005). The mean RBC of haemoparasite infected chickens were (2.9±0.055) higher than the non-infected chickens (3.0± 0.055) and was statistically significant (P<0.05). This result is in line with the report of Olayemi *et al*. (2014). This suggested that haemoparasites could reduce the hematology values of the chickens.

The mean TRBC, Hb, MCH and MCHC value of chickens infected with single haemoparasite was lower than chickens infected with more than one haemoparasites. Unevenly, the mean PCV and MCV values of chickens infected with single haemoparasite was higher than the chickens infected with mixed form of haemoparasite. Meanwhile, the TWBC count of chickens infected with single form of infection was similar to chickens infected with mixed form of haemoparasite. However, it was not possible to compare the present result with other research findings due to lack of further information.

Based on the existing study, the majority of chickens in the study area were raised local breed (71.7%) followed by cross (15.1%) and exotics breeds (Bovans brown) 13.2% with the average flock size of 4 chicken per house hold, respectively. This is similar with the report of Melkamu and Wube (2013) in DebsanTikura PA's, North Gondar Zone, who reported 93.3%, 4.06% and 1.9% indigenous, cross and exotic breeds, respectively. The present study is also in line with the survey of Halima (2007) in central Ethiopia with a flock size of 5 per house hold. However, as reported by Birhan (2016) in North Gondar zone the, flock size per house hold was 16.4. This might be due to variation in season of the year, availability of feed, incidence of diseases and presence of predators.

In the study area, 64.8% respondents replied that chickens are kept for the purpose of generating income by selling the chicken and their eggs, 25% of them said for only house hold consumption while 10.2% of them replied for both income generation and house hold consumption. Similar results were reported by Halima (2007) in central Ethiopia, 53.3% of the respondents stated that chickens were kept for the purpose of income generation while 19.2% of them said for house hold consumption.

In the current questionnaire survey, 14.9% of the respondents stated that parasitic diseases affected their chickens. The rest of the respondents (85.1%) believed that their chickens are affected by other diseases. The respondents observed that clinical signs such as diarrhea (70.3%), ruffled feather (25%)

and torticollis (4.7%) were common during chicken diseases. This could be due to non-hygienic and sanitary conditions of chicken houses and their surrounding area. A survey conducted in central part of Ethiopia, stated that around 72.4% of the respondents did not get proper examination and management health services; nevertheless, 6.7% of the farmers had consulting on chicken disease and management of health (Halima, 2007). It was expressed that in Africa one of the major problem in village chicken production is the dissemination of various infectious and non-infectious diseases (Gueye, 1998). A total of 77.8% of the respondents were using commercially available antibiotic and coccidiostate drugs purchasing from the nearby veterinary clinics and private rural drug stores to treat their sick chickens. However, 22.2% of the respondents reported that for the treatment of their sick chickens, they use traditional herbal drugs available around their areas (such as simiza, grawa and garlic). This might be due to lack of veterinary services around their areas.

The survey indicated that 68.2% of the respondents provided night shelter for their chickens in which humans and other animals' live (mixed shelter) while 31.8% respondents use separate shelters made for chickens. Similar study in central part of Ethiopia and North Gondar zone showed that 49.2% and 93.8% respondents provided mixed type of chicken shelter, respectively while 50.8% and 6.2% of the respondents provided a separate shelter for their chicken in central part of Ethiopia and North Gondar zone (Halima, 2007; Birhan, 2014).

From the present study, 72.2% of the interviewed respondents explained that their chickens are managed under extensive management system and the rest 27.8% said under semi-extensive management system. From the total interviewed respondents, 76.9% of them do not provide supplementary feed for their chicken. This result is in lined with the survey of Halima (2007) and Birhan (2014), who reported that 99.3%, 96.4% of the farmers supplied locally available source of feed by throwing on the surface of the ground, respectively. However, 0.72% and 3.3% of the chicken owners supplied the supplementary feeds.

5. CONCLUSSION AND RECOMMENDATIONS

In conclusion, haemoparasites of chicken are prevalent in the present study area. Four types of haemoparasites were identified of which *Aegyptienella* spp. was the most prevalent species. In addition, the hematological analysis showed that a lower mean value of blood parameter measurements were recorded in chickens infected with haemoparasites. Different risk factors such as species, sex, agroecology, breed, management system and origin can be considered as the risk factors associated for the occurrence of haemoparasites in chickens in the study area. Furthermore, Chicken haemoparasites were prevalent in midland areas than highland areas. From this study, it was possible to recognize that the majority of chickens were kept under extensive management system which leads them to become more exposed for haemoparasite infections. The findings revealed that haemoparasites are one of the most important chicken parasites in the study areas. The presence of chicken haemoparasites in the present study areas is a major burden to poultry growers and veterinary health professionals.

Therefore, from the above conclusion the following recommendations were forwarded:

- Efforts toward educating poultry growers about modern management systems and health care should get attention through continuous extension works to develop good management practices.
- For accurate diagnosis of haemoparasites and implement proper medication, consulting of veterinary professionals should be practiced. To control this economically important haemoparasitic disease of chicken, further studies should be conducted to formulate sustainable and cost effective prevention and control measures.
- The government, poultry growers and veterinary professionals should work in collaborate way of haemoparasite infection control.
- The chicken shelter should be cleaned, disinfected, sprayed with acaricides and insecticides if possible insect proofed house.
- Improving the hygienic and management system of backyard scavenging rearing style to lessen flock loss.

6. REFERENCES

Abdul, M., Begum, N., Dey, A.R., Paran, S. and Zahangir, M. A. (2014): Prevalence of Blood Protozoa in Poultry in Tangail**,** Bangladesh. *Journal of Agriculture and Veterinary Science*, 7(7): 55-60.

Adriano, E. A. and Cordeiro, N. S. (2001): Prevalence and Intensity of *Haemoproteus columbae* in three Species of Wild Doves from Brazil. *Mem Inst Oswaldo Cruz*. **96**: 175-178.

Alex, Z. (2009): Prevalence, Intensity and Pathology of Ecto and Haemoparasites Infections in Indigenous Chickens in Eastern Province of Kenya. MSc Thesis, Department of Veterinary Medicine, Kenya.

AL-zurfi, A.J. (2015): Prevalence of Haemoprotozoa of *Anas plathynchos* in Baghdad city, Iraqi. *Int. J. Dev. L. Sci*., 5(1): 1904-199.

Archawaranon, M. (2005): First Report of *Haemoproteus* Species in Hill Mynah Blood in Thailand. *Inter. J. Poul. Sci*., 4(8): 523-525.

Arsham, H. (2002). Descriptive Sampling Data Analysis. Statistical Thinking for Marginal Decision Making. Available March 10, 2014.

Atkinson, C.T., Thomas, N. T. and Hunter, D. B. (2008): Filaroid Nematodes in Parasitic Diseases of Wild Birds, C. M. Bartlett, Wiley-Blackwell. Pp. 439–462.

Ausraful, I., Anisuzzaman, Abul Kalam Mohammad Arifur Rabbi, Asadur Rahman, Md., Atiqul Islam and Hafezur Rahman, M. (2013): *Haemoproteus* Species Infection of Domestic Poultry of Bangladesh.

Birhan, M., and Getu, A. (2014): Chicken Production Systems, Performance and Associated Constraints in North Gondar Zone, Ethiopia. *J. Fisheries Livest Prod*., 2: 115.

Campbell, T.W. (1988): Avian Hematology and Cytology. Ames, Iowa State University Press. Pp.177-198.

Cannell, B.L., Krasnec, K.V., Campbell, K., Jones, H.I., Miller R.D. and Stephens, N. (2013): The Pathology and Pathogenicity of a Novel *Haemoproteus* Species Infection in Wild Little Penguins: Eudyptula Minor. *Vet. Parasitol*, 197: 74-84.

Cardona, C. J., Ihejirika, A. and McClellan, L. (2002): *Haemoproteus lophortyx* Infection in Bobwhite Quail.*Pub.Med*. *Avian Diseases*, **46**: 249–255.

CSA (2015a): Central Statistics Authority (CSA). Agricultural Sample Survey Report on Livestock and Livestock Characteristics. Addis Ababa, Ethiopia, Pp. 9-28.

CSA (2016. Central Statistics Authority (CSA). Agricultural Sample Survey Report on Livestock and Livestock Characteristics. Addis Ababa, Ethiopia, Pp. 37-95.

Donovan, T.A., Schrenzel, M., Tammy, A., Tucker, Allan, P. and Stalis, H. (2008): Hepatic Hemorrhage, Hemocoelom, and Sudden Death due to Haemoproteus Infection in Passerine Birds. *J. Vet. Diangn. Invest*. , 20(3): 304-13.

Emebet, E. (2017): Haemoparasite Infection in Indigenous Scavenging Chickens in and Around Bishoftu. DVM Thesis, University of Gondar, Faculty of Veterinary medicine, Gondar, Ethiopia.

Ferrell, S.T., Snowden, K., Marlar, A.B., Garner, M. and Lung, N.P., 2007: Fatal Hemoprotozoal Infections in Multiple Avian Species in a Zoological Park in Iraqi. *J. Zoo Wildl. Med*., **38**: 309-316.

Friend, M. and Franson, C. J. (2001): Field Manual of Wild Life Disease: General Field Procedures and Disease of Birds/Biological Resource Division, USA. Pp. 136-158.

Gimba ,F., Zakaria, A.,Mugok, L. B., Siong ,H. C., Jaafar, N., Moktar, M., Rashid, A. R., Amlizawaty, A., Abu, J., Sani, R. A., Amin-Babjee S. M. And Sharma R.S. (2014): Haemoparasites of Domestic Poultry and Wild Birds in Selangor, Malaysia. *Malaysian Journal of Veterinary Research*. , 5(1): 43-51.

Gueye, E. F. (1998): Village Egg and Fowl Meat Production in Africa. *World's poult. Sci.*, **54**: 73-86.

Hassan, R. H. (2015): Haemosporidian Parasites of *Gallus domesticus*, Poultry in Iraq. *International Journal of Advanced Research*, 3(8): 1046 –1054.

Halima, H. M. (2007): Phenotypic and Genetic Characterization of Indigenous Chicken Populations in North West Ethiopia. PhD Thesis. University of Free State, South Africa.

Iezhova, T.A., Dodge, M., Ravinder, N. M., Sehgal, N. M., Smith, T.B. and Valkiūnas, G. (2011): New Avian *Haemoproteus*. Holloway Avenue, San Francisco, California. *International Journal of Advanced Research.* 3(8): 1046-1054.

Ikhlas, A. M. and Marwa S. A. (2017): Prevalence of Pseudo-malaria (*Haemoproteus* spp.) Infection in Many Species of Birds. *International Journal of Research Studies in Biosciences*. , 5 (3): pp 11-14.

Klein, SL. (2004): Hormonal and Immunological Mechanisms Mediating Sex Differences in Parasite Infection. *Parasite Immunol*. , 6(7): 247-64.

Lapointe, D. A., Atkinson, C.T. and Samuel. M. D. (2012): Ecology and Conservation Biology of avian Malaria. Annuals of the New York Academy of Sciences. , 1249(1): 211–226.

Lawal, J.R., Bello1, A. M., Balami, S. Y., Dauda, J., Malgwi, K. D., Ezema, K. U., Kasim, M. and Biu, A. A. (2016): Prevalence of Haemoparasites in Village Chickens (*Gallus domesticus*) Slaughtered at Poultry Markets in Maiduguri, Northeastern Nigeria. *Journal of Animal Science and Veterinary Medicine*, **1**: 39-45.

Lloyd, S. (1983): Effect of Pregnancy and Lactation on Infection. *Veterinary Immunology and Pathology*, **4**: 153-176.

Maina, AN. (2005). Prevalence Intensity and Lesions Associated with Gastrointestinal Parasites of Indigenous Chicken in Kenya. MSc Thesis. University of Nairobi, Kenya.

Marzal, A., Ricklefs, RE., Valkiunas, G., Albayrak, T., Arriero. E. and Bonneaud. C. (2011): Diversity, loss, and gain of malaria parasites in a globally invasive bird. PLoS One, 6(7): e21905.

Melkamu, B.Y. and Wube, A. (2013): Constraints and opportunities of village chickens production in Debsan Tikuran Keble at Gondar Zuria woreda, North Gondar, Ethiopia. *International Journal of Scientific and Research Publications,* **3**: 9.

Merino, S., Moreno, J., Sanz, J. J. and Arriero, E. (2000): Are Avian Blood Parasites Pathogenic in the wild? A Medication Experiment in Blue Tits (*Parus caeruleus*). *Proc Biol Sci*., 267(1461): 2507-10.

Motta, R. O. C., Marques. M. V. R., Junior. F. C. F., Andery. D. A., Horta. R. S., Peixoto. R. B., Lacorte. G. A., Moreira. P. A., Leme. F. O. P., Melo. M. M., Martins. N. R. S. and Erika, B. M. (2013): Does Haemosporidian Infection Affect Hematological and Biochemical Profiles of the Endangered Black-fronted Piping-guan (*Aburria jacutinga*)? *Peer J.*, 1:45.

Murray, M., Trial, TCM. and Stephen, LE. (1983): Livestock Productivity and Trypanosomosis, ILCA, Addis Ababa Ethiopia. Pp.4-10.

Njunga, G.R. (2003): Ecto- and Haemoparasites of Chicken in Malawi with Emphasis on the Effects of the Chicken Louse, *Menacanthuscornutus* MSc Thesis, Royal Veterinary and Agriculture University, Denmark.

Olayemi, O.A., Jubril, A.J. and Adekola, A.A. (2014): Prevalence of Haemoparasites in Village Weaver (*Ploceus cucullatus*) in Ibadan, Nigeria. *J. World's Poult. Res.*, 4(4): 89-93.

Opara, M. N., Ogbuewu, I. P., Iwuji, C. T., Njoku, L., Ihesie, E.K. and Etuk, I. F. (2012): Blood Characteristics, Microbial and Gastrointestinal Parasites of Street Pigeons (*Columba livia*) in Owerri Imo State, Nigeria. *Scientific Journal of Animal Science,* 1(1):14-21.

Opara, M. N., Osowa, D. K. and Maxwell, J. A. (2014): Blood and Gastrointestinal Parasites of Chickens and Turkeys Reared in the Tropical Rainforest Zone of Southeastern Nigeria. *Open Journal of Veterinary Medicine*, **4**: 308-313.

Ozmen, O. and Haligur, M. (2004): A Study on the Presence of Leucocytozoonosis in Wild Birds of Burdur District. Department of Pathology, Faculty of Veterinary Medicine, Akdeniz University, Turkey. *Journal of Parasitology*, **97**: 682-694.

Opara, M. N., Oguobi F. C., Adiele E. E. and Jegede O. C. (2016): Survey of Haemoparasites and Hematology of Scavenging Ducks (*Anas Platyhyncha*) in Owerri Southeastern Nigeria. *J. Vet. Adv.*, 6(10):1325-1331.

Palinauskas, V., Valkiūnas, G., Bensch, S. and Bolshakov, V. C. (2011): *Plasmodium relictum* (lineage SGS1) and *Plasmodium ashfordi* (lineage GRW2): The Effects of the Coinfection on Experimentally Infected Passerine Birds. *Experimental Parasitology*. **127**:527-533.

Parker, PG., Whiteman, N.K. and Miller, RE. (2006): Conservation Medicine on the Galapagos Islands Partnerships among Behavioral Population and Veterinary Scientists. *The Auk.* , **123**: 625-38.

Pavlak, M. K., Vlahovic, J., Jarcic, A. D. and Zupancic, Z. (2005): Age, Sexual and Seasonal Differences of Hematological Values and Antibody Status to *Chlamydophila* spp. in Feral and racing pigeons (*Columba livia forma Domestica*) from an urban environment Zagreb, Croatia. *Eur. J. Wildlife Res*., **51**: 271-276.

Peirce, M. A. (1989): The Significance of Avian Hematozoa in Conservation Strategies in Diseases and Threatened Birds. ICBP J. E. Cooper, Ed., Helmsley, UK. Pp. 69–76.

Permin, A., Esmann, J. B., Hoj, C. H., Hove, T. and Mukatirwa, S. (2002): Ecto and Endo Haemoparasites in Free range Chicken in the Gomoronzi District in Zimbabwe. *Preventive Veterinary Medicine*, **54**: 213-224.

Poulsen, J., A. Permin, O., Hinbsbo, L., Yelifari, P., Nansen and Bloch. (2000): Prevalence and Distribution of Gastro-intestinal Helminths and Haemoparasites in Young Scavenging

Chickens in Upper Eastern Region of Ghana, West Africa. *Preventive Veterinary Medicine*, Pp: 237-245.

Sabunni, Z. A., Mbuthia, P. G., Maingi, N., Nyaga, P. N., Njagi, L. W., Bebora, L. C. and Michieka, J. N.(2011): Prevalence of Haemoparasites Infection in Indigenous Chicken in Eastern Province of Kenya. *Livestock Research for Rural Development*, 23:P. 238..

Sanni, A.A., Oyedokun, O. R. and Alaka, O.O. (2005): Preliminary Observations on Diurnal Rhythm in the Hematological Parameters of male African Giant Rats (*Cricetomys gambianus*, Water House). *Afr. J. Biomed. Res*., **3**: 117-120.

Sehgal, R. N., Gediminas. M., Tatjana, A. L. and Smith, T. B. (2006): Blood Parasites of Chickens in Uganda and Cameroon with Molecular Description of *Leucocytozoon schoutendeni* and *Trypanosoma gallinarum. Journal of Parasitology,* **92**: 1336-1343.

Sells, D. M., Hildebrandt, PK., Lewis, G. E., Nyindo, MB. and Ristic, M. (1976): Ultrastructural Observations on *E. equi* Organisms in Equine Granulocytes. *Infect. Immun*., **13**: 273-280.

Shadan, H. A. (2013): Prevalence of Blood Parasites in Local Chickens in Qaradagh District, Sulaimani, Iraq. *The Iraqi Journal of Veterinary Medicine*, 3(1): 17–21.

Soulsby, E.J.L. (1982): Helmints, Arthropods and Protozoa of Domesticated Animals, Bailliere Tindall, London, Pp. 703-705.

Svensson-Coelho, M., Ellis, V. A., Loiselle, B. A., Blake, J. G. and Ricklefs, R. E. (2014): Reciprocal specialization in Multihost Malaria Parasite Communities of Birds: a Temperate-tropical Comparison *Am. Nat*., **84**: 624-635.

Tadelle, D., Million, T., Alemu, Y. and Peters, K. J. (2000): Village Chicken Production System in Ethiopia: use Patterns and Performance Evaluation and Chicken Products and Socio-economic Functions of Chicken. Debrezeit Agricultural Research Center, Debrezeit, Ethiopia. (Available at, http:// www. Cipav. Org. co/irrd/irrd15/1/tade b/5/. htm. Accessed on: June 10, 2016).

Tarello, W. (2005): *Trypanosoma avium* Incidence, Pathogenicity and Response to Melarsomine in Falcons from Kuwait. *Parasite: Pub. Med*., 85-7.

Thrall, M.A. (2004). Veterinary Hematology and Clinical Chemistry. Williams and Wilkins, Philadelphia, P. 518.

Thrusfield, M. (2005): Veterinary Epidemiology 3rd ed. UK: Blackwell Science Ltd., A Blackwell Publishing Company, P. 233.

Valkiunas, G. (2005): Avian Malaria Parasites and Other Haemosporidia. CRC Press, Boca Raton, Florida; USA. P.946.

Valkiunas, G., Iezhova, T. A., Carlson, J. S. and Sehgal, R. (2011): Two new *Trypanosoma* species from African Birds with notes on Taxonomy of Avian *Trypanosomes*. *J. Parasitol., doi*. , 10, 1645/GE, 2796.1.

Wettere, V. and Arnaud, J. (2013): Merck Veterinary Manual. Overview of Blood Borne Organisms in Poultry (Available at: http://www.merckmanuals.com/vet/poultry/bloodborneorganisms. Accessed on: December 12, 2016).

WGARDO, (2015): West Gojjam Zone Rural and Agricultural Development Office (.WGARDO) Annual Report of Work Plan Implementation Program.

William, F. L. (1995): Poultry Pest Management. Ohio State University, Columbus, Ohio, USA. P. 853.

William, R.B. (2005): Avian Malaria: Clinical and Chemical Pathology of *Plasmodium gallinaceum* in the Domestic Fowl, *Gallus domesticus*. *J. Avian Patholo*. , **34**: 29 – 47.

Zidkova, L., Cepicka, I., Szabová, J. and Svobodová, M. (2011): Biodiversity of Avian Trypanosomes; Department of Parasitology, Faculty of Science, Charles University in Prague, Prague: Czech Republic, 7(128): 44.

7. ANNEXES

Annex **1**: Blood Sample Collecting Techniques (Campbell, 1988).

1. Hold the chicken horizontally on its back. The assistant uses one hand to hold the legs and places the other hand under the back to support the chicken.
2. Pull a wing of the chicken out towards you.
3. Note the wing vein, clearly visible running between the biceps and the triceps muscels. The wing vein forms a V (bifurcates). Note the tendon of the pronator muscle that run across the V.
4. Pluck away any small feathers that obscure the vein.
5. Disinfect the area around the bleeding site by swabbing with 70% alcohol.
6. Insert the needle under tendon. Direct the needle into the wing vein in the direction of the flow of blood. Do not insert the needle too deeply. Keep clear of the ulnar nerve.
7. Once the tip of the needle is in the vein, gently pull the plunger of the syringe. Blood will flow into the syringe. If blood does not flow, release the plunger and make a very slight adjustment to reposition the end of the needle.
8. Be patient and use a gentle suction to withdraw the blood. Chicken veins will collapse readily.
9. If a hematoma forms, try bleeding from the other wing.
10. After removing the needle, apply pressure to the vein for a few seconds to discourage further bleeding.

Annex 2: Preparation of Thin Blood Smears (Campbell, 1988).

1. Place a drop of blood approximately 4 mm in diameter on the slide.
2. Spread the drop by using another slide (spreader), placing the spreader at an angle of 45^{o} and backing into the drop of blood. The spreader catches the drop and it spreads by capillary action along its edge. Now, push the spreader across the slide; this pulls the blood across to make smear.
3. Smears should be air-dried, and then dipped into 100% methanol. Slides can be stored in a small plastic slide box or stain with Giemsa.

Annex 3: Staining of Blood Smears (Campbell, 1988).

1. The smears will be fixed with 100 % methanol for ~3' and rinse off in tape water.
2. The dried/fixed smear will be stained with 10% Giemsa for ~30'.
3. Rinse off the slide in tape water and dry thoroughly using by blotting paper.
4. View slide on under oil immersion with a 1000× objective.

Annex 4: Process of Automated Hem Analyzer

1. The test tube with blood sample moved to the hem analyzer circular cartridges that rotate to be made the sample available.

2. After a few seconds the automated hem analyzer display the result as printed in the labeled paper.

Annex 5: Data Collection Format Sheet

ID No	District	Agroecology	PA,s	Age	Sex	Breed	Management system	Result
1								
384								

Annex 6: Parasite Identification and Hematology Result Record Format Sheet

ID no	Identified Parasite	TRBC	TWBC	PCV	Hb	MCV	MCH	MCHC

Annex 7: Questionnaire Format

QUESTIONNAIRE

UNIVERSITY OF GONDAR COLLEGE OF VETERINARY MEDICINE AND ANIMAL SCIENCES

Studies on management, housing, rising, feeding and medication information of poultry in the three districts of Mecha, South Achefer and Bahirdar Zuria.

Questioner format

Name_____

Sex: Male_____ Female _____Age_____

District_____Kebele_____zone_____

1. Why do you raise chicken?

A. Cash income B. for consumption C. Cash income and Consumption

2. What type of chicken breed you raise?

A. Local B. cross C. exotic

3. How many poultry do you have?

A. Local; Male_____ Female_____

B. Cross; Male_____ Female_____

C. Exotic; Male_____ Female_____

4. Formulation of chicken feed ration

A. mixing of cereals B. Scavenging freely

5. What kind of feed do you prepare for your chicken?

A. Cereals only B. injera C. mixed feed

6. How do you give the feed and water to poultry?

A. on feeding trough B. by scavenging

7. Housing system of chicken

A. separated B. Mixed type

8. What type of drug do use when the chicken became sick?

A. Herbal /using plants B. commercial available drugs

9. What type of disease affects your poultry? What are the common symptoms?

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